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COMPARISON OF PHOTON COUNTING DEVICES USING ANTIBIOTICS AND HEAVY METAL BIOSENSORS

Master of Science Thesis

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Abstract

Bioluminescence has been of great interest for the past few decades. There have been plenty of biotechnological methods to detect, estimate, view and establish the bioluminescence obtained from variety of organisms. Due to the advancements in genetic engineering technologies, increasing number of instruments has been manufactured for the detection and counting of bioluminescence. However, very few studies have been reported comparing the efficiency and working of different photon counting instruments available today. Henceforth, special effort has been taken to study and compare the photon counting devices in this thesis.

The aim of the thesis is to compare three different photon counting devices available at the laboratory of environmental engineering and biotechnology; namely Chameleon Multilabel plate reader from Hidex Oy, Victor^2 Wallac from Perkin Elmer life sciences and IVIS Lumina Xenogen from Caliper Life Sciences. The efficiency of these three devices were compared by using two different bacterial strains, one is bioluminescent whole-cell biosensor strain, *Escherichia coli* DPD2794 and the other being an arsenic biosensor strain *Escherichia coli* XL-1(parsRluxCDABE). These Genetically engineered bacterial strains have been grown under laboratory conditions and comparative luminescence was done by bioluminescence measurement methods and agar diffusion method (bio-photonic imaging station). Inducible ciprofloxacin with different concentrations were used for *Escherichia coli* DPD2794 and arsenite $NaAsO_2$ (III) and arsenate Na_2HAsO_4 (V) were used as a biosensor for detecting the amount of bioluminescence emitted from respective bacterial species. On comparing the efficiency of bioluminescence detected by three different photon counting devices, Chameleon was found to provide most sensitive and IVIS the lowest. However all the approaches is useful depending upon the application employed.

Preface

This study was conducted at the Department of Chemistry and Bioengineering, Tampere University of Technology. I am grateful to my supervisor, Researcher Ph.D. Anna-Liisa Välimaa and examiner Professor Matti Karp for their encouragment and guidance througout the thesis study. I thank Senior Researcher Ville Santala for all the help and guidance in proceeding with the experiment. I take this opportunity to thank Rahul krishnan, Bobin George and Anne Rantala for helping me with laboratory work.

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Tampere, January 2011

Daniel Praveen Paul

Contents

Abbreviations

AAS	Atomic absorption spectroscopy
ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
BART	Bioluminescence assay in real-time
BL	Bioluminescence
BRET	Bioluminescence resonance energy transfer
CAM	Chloramphenicaol-transacetlyase
CCD	Charge coupled device
CFX	Ciprofloxacin
CL	Chemiluminescence
IARC	International agency for research on cancer
MS	Microsoft
OD	Optical density
PEG	Poly ethylene glycol
ROI	Region of interest
UBL	Quantum Yield
UC	Chemical yield
UEX	production yield
UF	emission quantum yield
WHO	World Health Organization

1 Introduction

Biological emission of light has fascinated mankind for millennia. Bioluminescence is a naturally occurring chemical phenomenon that has been studied for many years. In this process, at least part of reaction products are obtained in an electronically excited state, which can either decay, emitting photons of visible light or act as sensitizer (Meighen, 1991). Bioluminescence is found in many living organisms like jelly fish, firefly, common glow worm microorganisms, etc..,

Scientists and researcher used the fantasy to their advantage and started studying natural luminescence. All Bioluminescent proteins use molecular oxygen to oxidize their substrates to a product. These Bioluminescent proteins are present for bacteria, firefly and fungi showing various emission ranges. Molecular biology techniques are used extensively to improve the existing bioluminescent proteins and they are called as Mutant proteins.

However, the detection of the bioluminescence and the measurement of amount of bioluminescence had to be detected to evaluate and establish certain scientific experiments. Luminescence based techniques either relying on CL or BL systems offer an undoubted advantages over other optical detection techniques. Due to the advancements in technologies, increasing number of instruments has been manufactured for the detection and counting of bioluminescence. However, very few studies have been reported comparing the efficiency and working of different photon counting instruments available today. Henceforth, special effort has been taken to study and compare the photon counting devices.

There are two types of devices that are involved in this thesis and the first one is Luminometers. Luminometers are simple instruments designed to perform high intensity light measurements in a wide range of analytical formats. The luminometers have a large range of measurement from single tube to over thousand wells in a micro titer plate. Broad spectrums of optical filters are available for luminometers. (Foord et al., 2000)

On the other hand high sensitive imaging systems with ultra sensitive charge coupled device (CCD) are available. In this type of lumino-graphs the signal to noise ratio is extremely low compared to that of the luminometers. Imaging stations have 3D viewing which is at the moment used in advanced laboratories. These CCD detectors allow real time acquisition of BL signals. 3D technology allows the intact study of animals; it also gives a wide range of applications.(Meyer & Kirkland, 2000)

The aim of the thesis is to compare three different photon counting devices available at the laboratory of environmental engineering and biotechnology; namely chameleon multilabel plate reader from Hidex Oy, Victor^2 Wallac from Perkin Elmer life sciences and IVIS lumina Xenogen from Caliper Life Sciences. These instruments were studied individually and their comparison was not studied before. The efficiency of these three devices were compared by using two different bacterial strains one is bioluminescent whole-cell biosensor strain, *Escherichia coli* DPD2794 and the other being an arsenic biosensor strain *Escherichia coli* XL-1(parsRluxCDABE). These bacterial strains are used in the experiment to compare and analyze the different parts of the experiment. Three photon counters are to be effectively analyzed using the bacterial strains mentioned above.

2 Background

2.1 Bioluminescence

Bioluminescence (BL) is simply light produced by a chemical reaction in a living organism (Roda et al., 2009). The biological emission of light has fascinated humankind for millennia. The biochemistry of light production is certainly well understood Bioluminescent organisms are widely spread all over the environment and they are remarkably diverse set of species (Meighen, 1991). However bacterial bioluminescence has been of great interest for molecular biologists and biotechnologists. Significant differences are seen between the bioluminescent strains and the structures of the luciferase (enzyme) and luciferin (substrates) (Meighen, 1991). Almost all the luminescent bacteria have been classified into three genera namely vibrio, photo-bacterium and xenorhabdus (Ochman & Wilson, 1987).

These phenomena of bioluminescence have been of great interest for the past two decades because of its wide range of research applications and inter-disciplinary approach to establish healthy and strong future applications. Bioluminescent bacteria are one of the important criteria to be taken into consideration for the production of ATP. The discovery and characterization of cis-acting regulatory proteins have been used in reporter gene fusions and the frequently used ones in bacterial systems are lac Z (β- galactosidase), phoA (alkaline phosphatase), cam (chloramphenicaol-transacetlyase), and lux (bacterial luciferase). The utilization of five gene luxCDABE reporter system allows continuous monitoring of light emission. The main advantage with the lux reporter system is the sensitivity and large dynamic range of light detection equipment (Meighen, 1991).

Bioluminescent bacteria are found in marine and terrestrial environments. The bacterial lux genes in proteins exhibit variable thermo stability. They do not efficiently function in standard media at growth temperatures, typically for many other bacteria.

The overall intensity of bioluminescence depends on the quantum Yield ((UBL), which is the product of the chemical yield of the reaction (UC), the excited state production yield (UEX), and the emission quantum yield of the excited state (UF). When compared with conventional CL systems, the peculiarity of a BL reaction is the much higher efficiency of light emission process. The advancements of molecular biology in the last decades have further enlarged the diffusion of BL-based bio-analytical methods. Bioluminescence resonance energy transfer (BRET) assays for monitoring protein-protein interactions, BL whole-cell biosensors for the detection of heavy metals and xenobiotics, and whole-body

BL imaging systems for tracking tumor cells and evaluating gene expression in living animals are representative examples of recent BL applications with a largely unexplored potential (Roda et al., 2009).

Most bioluminescent proteins are enzymes (luciferases) that catalyze the oxidation of their substrates (luciferins). There are N numbers of bioluminescent proteins and they have their own applications. Their names and applications are mentioned below in the table.

Table 1. Properties of wild-type bioluminescent (BL) proteins employed in bio analytical applications (Roda et al., 2009)

BL protein	Organism	Reporter gene	MW (kDa)	BL λ_{max} (nm)	Substrate; other required components
		Luciferases			
Bacterial luciferase	*Vibrio, Photobacterium, Xenorhabdus* genera	*lux*	80 (dimer)	490	Aliphatic aldehyde; $FMNH_2$, O_2
Firefly luciferase	*Photinus pyralis*	*Fluc*	61 (monomer)	557	D-luciferin; ATP, Mg^{2+}, O_2
	Luciola italica	*Lit*	61 (monomer)	566	D-luciferin; ATP, Mg^{2+}, O_2
	Hotaria parvula	*Hluc*	61 (monomer)	568	D-luciferin; ATP, Mg^{2+}, O_2
Renilla luciferase	*Renilla reniformis* (sea pansy)	*Rluc*	36 (monomer)	480	Coelenterazine[a]; O_2
Railroad-worm luciferase	*Phrixotrix hirtus*	*PxLuc*	60 (monomer)	623	D-luciferin; ATP, Mg^{2+}, O_2
Gaussia luciferase	*Gaussia princes* (marine copepod)	*Gluc*	20 (monomer)	480	Coelenterazine[a]; O_2
Metridia luciferase	*Metridia longa* (marine copepod)	*MLuc*	24	480	Coelenterazine[a]; O_2
Vargula luciferase	*Vargula hilgendorfii* (marine ostracod)	*Vhl*	62	465	Coelenterazine[a]; O_2
Cypridina luciferase	*Cypridina noctiluca* (marine ostracod)	*Cnl*	61	465	Coelenterazine[a]; O_2
		Photoproteins			
Aequorin	*Aequorea victoria* (jellyfish)	*Aeq*	22 (monomer)	469	Coelenterazine[a]; Ca^{2+}
Obelin	*Obelia longissima* (hydroid)	*Obe*	22 (monomer)	485	Coelenterazine[a]; Ca^{2+}

The bioluminescent proteins are bacterial luciferases, firefly luciferases, renilla luciferases, aequorin, etc., however the organisms varies according to the types of bioluminescent protein. The molecular weight varies according to the bioluminescent protein, beetle luciferase, having the higher molecular weight. The firefly luciferase/luciferin reaction is given below in Figure 1.

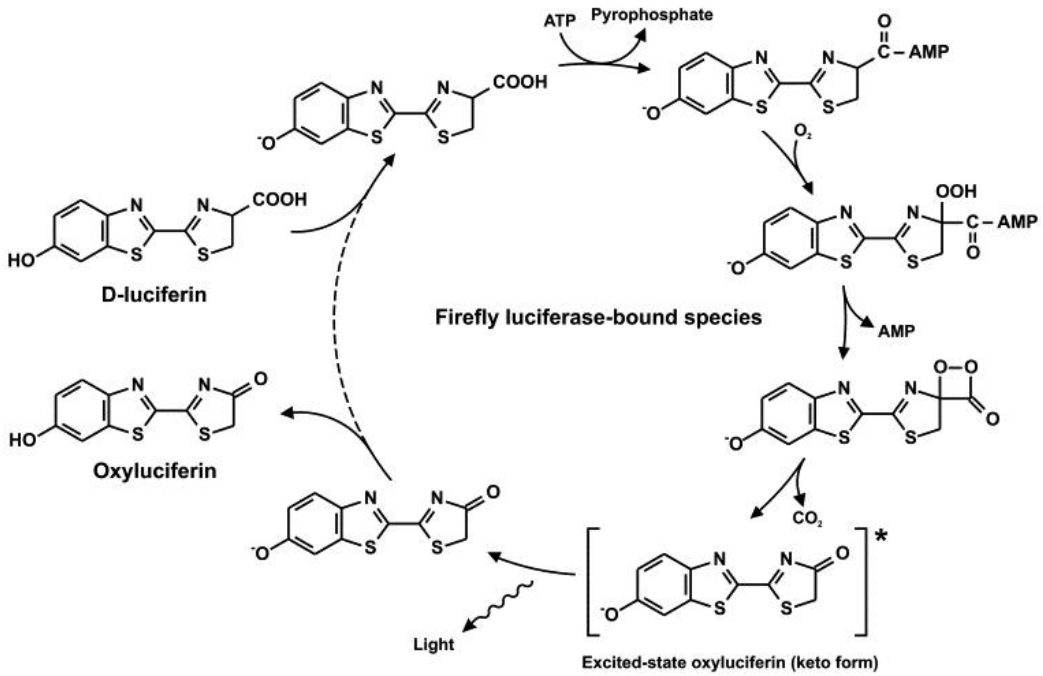

Figure 1 *the firefly luciferin/luciferase bioluminescence reaction: firefly luciferase catalyzes the reaction of D-luciferin, ATP and oxygen through the formation of D-luciferyl adenylate and a high-energy dioxetanone intermediate to give singlet excited-state oxyluciferin* (Roda et al., 2009)

In the above picture the reaction firefly catalyzes D-lucifern and oxygen through a D-luciferyl adenylate and a high-energy dioxetanone intermediate to give single excited state oxyluciferin. The bioluminescent protein (firefly) was one of the early found BL proteins that have been in research and in use for more than three decades. Their structure and morphology are being investigated for over a period of twenty five years. Understanding the chemistry behind the individual structural morphology makes it approachable in molecular biological researches.

Another important and widely used BL protein is the bacterial luciferase. Because of its well known structure and easily reconstructed, it has been of a great interest for scientists, molecular biologists and researchers. Bacterial luciferases have higher molecular weight compared to any other BL proteins. Their molecular weight is 77 (dimer). The reaction that catalyzes the bacterial luciferase is given below in Figure 2.

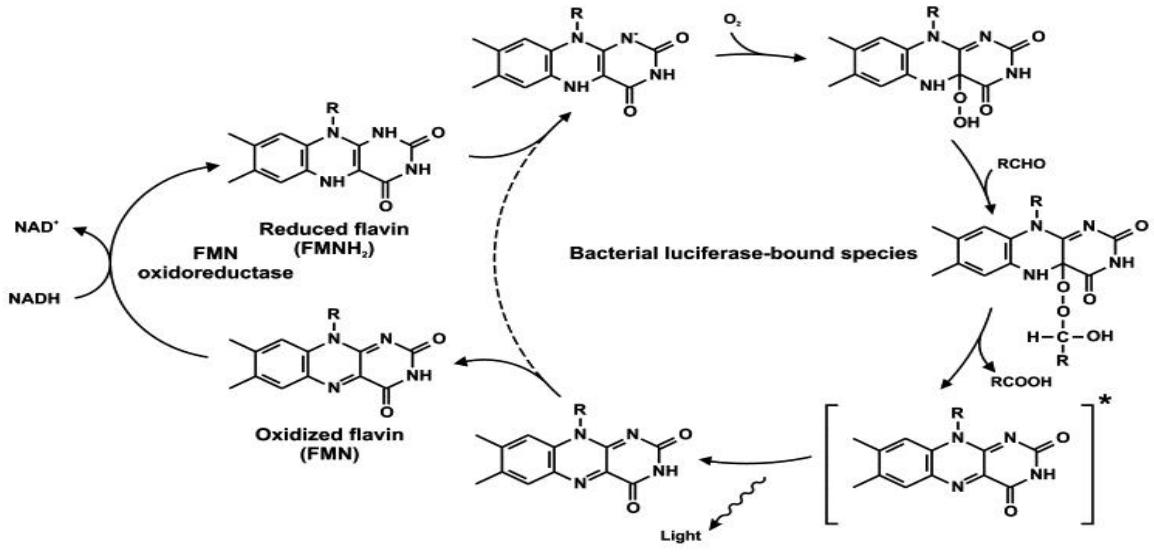

Figure 2 *The bacterial luciferase bioluminescence reaction: reduced flavin bound to the bacterial luciferase is deprotonated at nitrogen, then it reacts with oxygen and bacterial luciferin. Elimination of a carboxylic acid leads to an excited-state oxidized flavin* (Roda et al., 2009)

In spite of the well known structural morphology and chemistry behind BL proteins, their luminescence has to be detected using various luminescence counters and detectors. In recent years the application of bioluminescent protein has quadrupled in emerging and high end technologies and machine oriented applications.

2.2 Visualization of Bioluminescence

There are an increasing number of specialized instruments that may be used for the purpose of measuring bioluminescence. There are several luminescence counters visualization machines to detect and count the luminescence produced. These instruments have been used to detect bioluminescence in a number of organisms using either bacterial luciferases lux (Roda & M or firefly luciferases luc (Roda et al., 2009) as reporters. Dynamic measuring range of the newer luminometers ranges from $10^6$to 10^8logs. In new luminometers, features like agitation, temperature control is a must and in older luminometers, they came at extra cost. The newer luminometers come with customized software that designs the program and makes the experiment user friendly and beneficial.

Sample containers also have become specialized. In the case of multi-label luminometers there are specialized micro-titer plates to avoid the cross talk. Black plates with transparent bottom and complete white opaque plates to increase the intensity in low luminescent proteins are being used. On the other hand, it is also possible to measure and document bioluminescence without purchasing a dedicated instrument. In most laboratories, equipments and supplies that can be used successfully in many applications already exist. There are certainly limitations to their sensitivity; especially since these instruments were usually designed with some other applications in mind. BART-bioluminescence assay in real-time is measure in bioluminescence.

2.2.1Chameleon

Chameleon multi-label plate reader from Hidex Oy is analytical device that serves as an ideal tool for the researchers. Reliable and authenticated results can be obtained when and where they need them. Advanced technology being implied in Chameleon reader makes it ideal for scintillating counting, luminescence, absorbance and fluorescence readings. Own settings can be made with the Microwin data analysis software, which comes with the Chameleon multi-plate reader from Hidex Oy (Ukonaho et al., 2007). The software allows shifting between luminescence fluorescence and absorbance and also the time intervals between each reading. Temperature control is another feature that is found in Chameleon multiplate reader. The readings can be exported as MS excel or other data reduction programs.

Less complexity of the program makes Chameleon easy to use and use it over a long period of time. However it is a multi-plate reader which can only detect or read luminescence and it cannot visualize the reaction that is going on inside. To visualize the reaction going on inside the microtitre plate an imaging system has to be used. Chameleon has a photo

multiplier tube which is used in intensifying the low level luminescent signals.. The photo multiplier tube is a device which incidents and electromagnetic field ejects photo-electrons from a photo-cathode which is multiplied by a cascaded secondary emission process to produce pulse of charge at the anode (Foord et al., 2000). At high light levels these pulses will overlap and a measurement of anode current gives the required incident light intensity; at lower light levels, however the greatest amount of information is obtained when the photomultiplier is used in such a way that the individual photoelectrons from the photocathode is detected.

Many photomultiplier tubes of an advanced type have been designed for use, together with scintillators, as detectors in Bioassays and nuclear physics experiment. However, the uses of Photomultipler tubes are not just restricted to bioassays and nuclear physics, its application is interdisciplinary and nowadays photomultiplier tubes are coupled with many other instruments for high efficiency.

2.2.2 Victor^2 Wallac

Wallac 1420 multilabel counter from Perkin Elmer is an innovative system for quantitative detection of light-emitting or light-absorbing markers. It is suitable for flash or glow luminescence, fluorescence, high-sensitivity time resolved fluorescence, flash absorbance and photometry (Mosaddik et al., 2004). Victor^2 Wallac multilabel plate readers run multiple assay technologies on a single platform and are designed to support current and future demands. Victor is known for its speed and performance in screening labs and drug discovery labs.

The usage of bioassays has been practiced over three decades and the companies trying to produce multilabel plate readers are improving the performance of the machines by combining different technologies available in market. The outcome of the combination of technologies is remarkable and it has been helping the researchers and scientists to try out new combination and possibilities. Nevertheless, Wallac Victor^2 is one such instrument which is used in the preparation processing and continuous updating of the machine for the past 20 years. Selection of wells from the micro-titer plates and automatic/repeat program makes it easier for the researchers to take reading over a long period. Sleek design and simple load in program makes it easy to use. The combination of fluorescence and bioluminescence with color indicators, allows the viewer to have an idea of the luminescence/fluorescence range from the very initial moment.

2.2.3 Xenogen

In recent years Invivo imaging of animals and micro-organisms has obtained great interests amongst scientists and technologists. 'Invivio optical (Biophotonic) imaging is bases on gathering and processing information encoded in signals that are generated as a result of interaction of light photons with cellular components that make up the living tissue' (Pomper, 2008). The Biophotonic imaging station used for the experiment is (Xenogen VIVO Vision IVIS Lumina, Caliper Life Science System, USA)

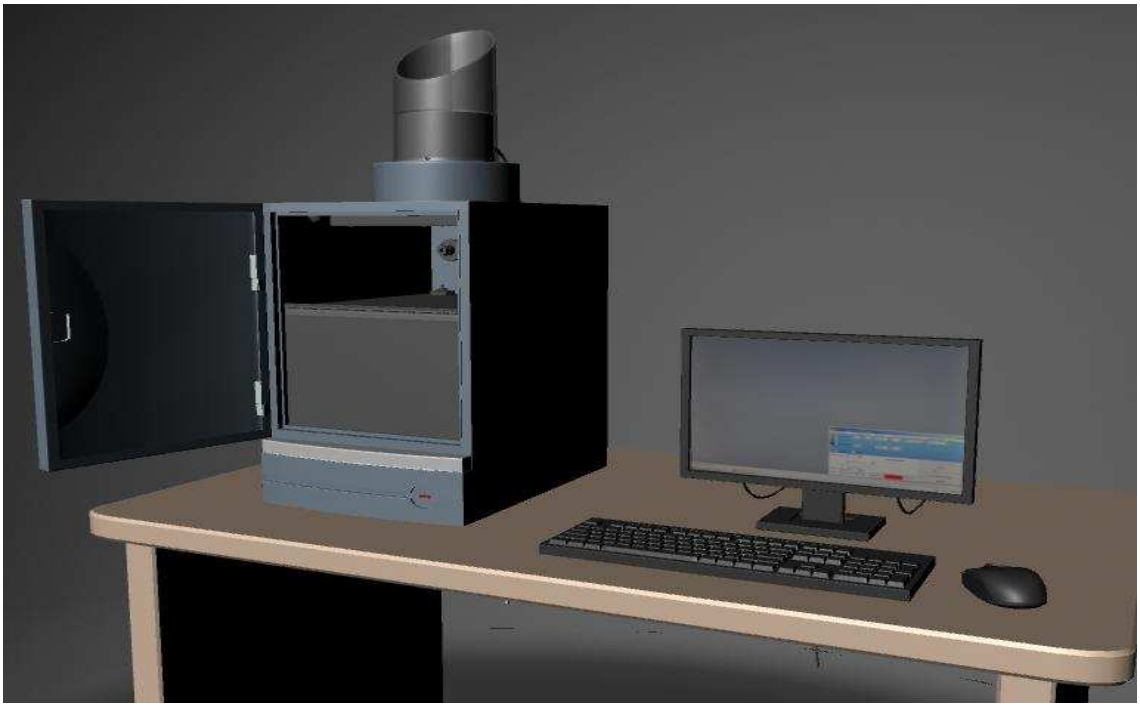

Figure 3 *IVIS Lumina- Xenogen, Caliper life sciences, representation of a Biophotonic imaging station*

IVIS Lumina Xenogen has a CCD (Charge coupled Device) Camera, capable of capturing sequences of low-light level images. The camera can be cooled to around -90°C. This is the special advantage of a charge coupled device camera. This helps in the imaging method used for bioluminescence and fluorescence (Jenkins et al., 2003). IVIS means in-vivo imaging system (Hsieh et al., 2009). The Advancement in compiling the CCD camera technology with in-vivo imaging has caused a great deal of improvement to the field of bio-imaging and this applies to Xenogen too.

Live visualization of laboratory animals like mice and rabbits and microorganisms like *E.coli, Staphylococcus aureus* helps in the immediate visualization of the result and better understanding of the mechanism going on in the animals or bacteria. The compelling usage of software makes the analysis of the image from the experiments easier and quicker.

Bioluminescent and photographic image from IVIS lumina Xenogen can be seen below in the Figure 4.

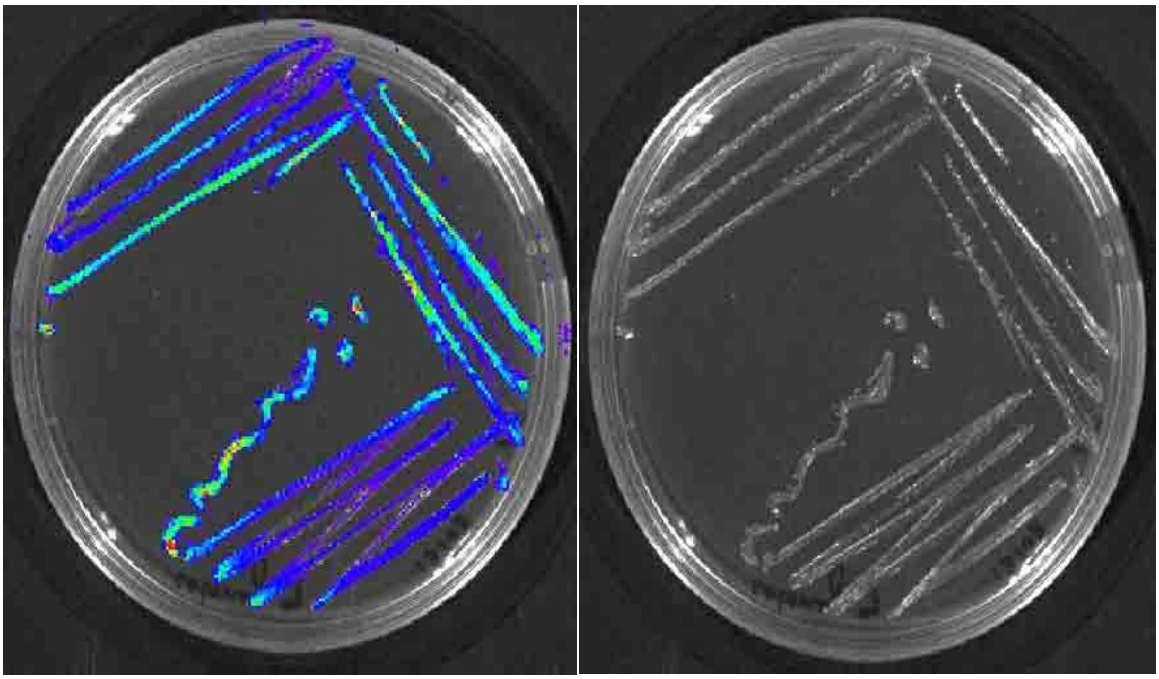

Figure 4 *Luminescent and photographic image taken from IVIS Lumina Xeongen*

The Figure 4 was obtained using IVIS Lumina Xenogen from Caliper Life Science in the Labortatory of Environmental Engineering and Biotechnology .The bioluminescent images from Petri-plates were obtained as photons from IVIS lumina Xenogen (Akens et al., 2010). Biophotonic imaging station has floor which can be adjusted to various heights (A, B, C, and D), floor height determines the output size of the image. Floor size D has the lowest output image size, whereas A has the highest output image size. However, the detail of the image still remains the same. Four halogen lamps are present on the top of the inner Xenogen body. These lights are useful when worked with live animals. Opening of the camera shutter and the imaging time can be adjusted from living image 3.1 software. Post processing of the image is done using imaging software.

Post processing of the software with living image is easy to use. It allows adjusting the brightness/contrast of the output image. ROI (Region of Interest) tool with auto all and manual drawing tools allows one to select the region of interest and calculate the luminescence/fluorescence produced from that particular region. Hence specific areas can be calculated if required for the experiment. Length and width measurement tools are useful to calculate the length of the luminescent field. Histogram and wave charts helps to find the luminescent range in the form of a line graph.

2.3 Heavy metals

2.3.1 Arsenic

Arsenic is a steel grey metal like material present in the earth's crust. It is odorless and usually colourless substance (Baumann & van der Meer 2007). It is the thirty third element in periodic table, which also means poison (Rosen, 1999). It is usually released in the air by volcanoes through minerals and ores and also due to industrial processes. It is mostly combined with sulphur, oxygen and chlorine elements and in industries it occurs during the smelting process of lead, gold, zinc, cobal and nickel. It has an atomic number 33 and its compounds are used as pesticides, insecticides and herbicides. Arsenic is a naturally occurring substance on the face of this earth just like lead, mercury or most commonly known asbestos. These are substances that we come across very often in our day to day life.. It is also an important component in the preparation of wood preservatives. As arsenic is highly immobile in the arena of agriculture, it tends to stay in the top layers of the soil. Another main source of arsenic contamination is through underground water

Arsenic contamination reaches various water streams through the waste, volcanoes and other ores. Arsenic eventually ends in lake, pond and also in sea waters, making the lives of fish and human beings in a potential risk. At low pH values of arsenate is sorbed in the soil more strongly than arsenite. The ionic strength decreases the binding of arsenate but not arsenite oxyions (Smith et al., 1998)

Arsenic is a mobile element that circulates in various forms through the atmosphere, water, and soil before ending up in the natural sink, bottom sediments (Lepp, 1981). Arsenic originates from natural and anthropogenic sources, viz., industry, mining, farming, weathering of rocks, and atmospheric deposition (Smith et al., 1998). This way arsenic is either flown by wind, inhaled by us or it mixes with the food substances and enters our system. There are possibilities for arsenic poisoning when a person works in a place which involves exposure to arsenic. Arsenic is naturally found in earth crust and is distributed extensively in the form of minerals like arsenolite, orpiment and realgar. The exposure to inorganic arsenic has been seen particularly in the recent studies for the detection of human diseases (Mushak & Annemarie, 1995).

Arsenic is widely distributed in the Earth's crust and occurs primarily in four oxidation states: arsenate [As (V)], arsenite [As (III)], elemental arsenic [As (0)], and arsenide [As (–III)]. Microbes play an immense role in transformation of various oxidation states in arsenic. Inorganic arsenate entering the microbial cytosol through the phosphate transport system is reduced to arsenite, which is then extruded out of the cell (Rosen, 1999). Arsenite is also generated by certain microbes that use arsenate as the terminal electron acceptor in anaerobic respiration (Heimann et al., 2007). Microbes can also convert

inorganic arsenic into gaseous methylated arsenide (Bentley & Chasteen, 2002). However, whether microbes can metabolize arsenic salts to elemental arsenic remains to be determined. Marine microorganisms can convert inorganic arsenic to various water- or lipid-soluble organic arsenic species.

Arsenic has two important oxidation states one is pentavalent (As (V)) and the other one is trivalent (As (III)). In solution the pentavalent form, H3AsO4, exists as the oxyanion arsenate. As a solid, the unhydrated trivalent form is arsenic trioxide (As2O3). Resistance to arsenic can be seen in both gram positive and gram negative bacteria. The uptake mechanism of arsenic is shown below in figure 2.

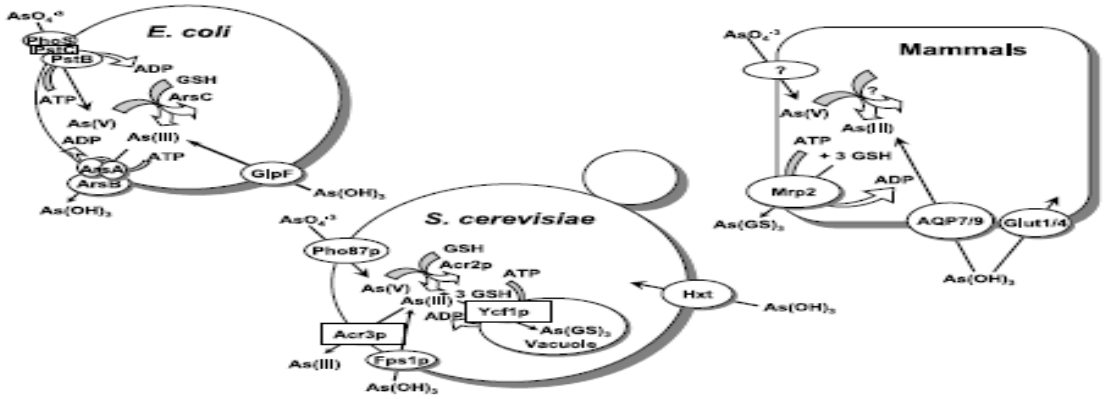

Figure 5 *Uptake Systems for Metalloid Oxyanions and Oxyacids. Uptake of arsenic in E.coli, S.cerevisiae and mammals are show above in the figure* (Rosen, 1999)

Figure 2 above shows the regions of Bangladesh (Paul, 2004) and India (Pandey et al., 2002) that are contaminated with arsenic. These regions are contaminated with arsenic more than any part of the world. Thus, the people living in this area are more proned compaered to any other parts of the world. Existence of arsenic contaminated ground water has been found in many countries like Chile (Dittmar, 2004), Mexico, Taiwan and Argentina. Yet it was discovered that, the West Bengal population of Indian Subcontinent has been subjected to the maximum of arsenic contamination (Baumann & Van Der Meer, 2007) compared to any other country. Studies reveal that about 800 000 people in that region by the year 1994 have been exposed to the ill effects of arsenic contaminated ground water as they utilized water from tube wells that were installed in 1960's.

Arsenic contamination causes potential health damages for animals and human beings as well (C. Liu et al., 2010) (Duker et al., 2005). This arsenic contamination enters human through land, water and air (Zhang & Wang, 2004). The exposure of arsenic cause adverse effects in human beings and animals. Most of the toxic effects arise from the inorganic arsenic and the number of death occurring by inhaling arsenic rises with the amount of intensity and the exposure towards arsenic. Its toxic effects are cancer which affects lungs,

skin, bladder, kidneys and liver. Skin cancer due to inorganic arsenic takes prolonged time to develop. Nervous system disorder can take place by exposure to arsenic. Abortions and congenital malformations are also a cause of arsenic exposure. Irritation in the nose, throat and lungs and gastrointestinal problems are also problems of arsenic. It has high toxic effect on skin causing pigment change and thickening of skin. Acute arsenic also causes internal bleeding and inflammation of the heart (Mushak & Annemarie, 1995).

2.3.2 Mercury

Mercury is mostly emitted from natural sources and it is one of the most persistent pollutants. It is usually found in the form of atmospheric Hg which has a life time for almost 2 years. The problem with mercury is usually identified when consumed in the form of fish and other marine products. Mercury can also be released due to natural causes like volcano, water bodies etc. (Ferrara et al., 2000). The maximum level of mercury emission is found to occur from fossil fuels such as coal and boilers. China stands the highest position in its contribution for mercury emission followed by other Asian counties such as India, Japan etc. Due to the fact that Asia is the highly populated continent and also that it has seen a considerable increase in the economic growth, the increase in mercury has elevated to a higher level posing serious risk to the environment and mankind. (Li et al., 2009)

Mercury has special properties which enable it to be used in several industries such as production of batteries, thermometers, fluorescent lamps, electronic switches etc. The industry that uses mercury at an elevated level would be chemical industry. The discharge from these industries emits extreme levels of mercury into the aquatic streams and hence causes risk to the aquatic system. More than industries, the mining areas are the best contributors towards mercury contamination. Asian countries possess the high level contamination sites of mercury. Certain examples of mercury found in high levels are Wanshan Hg mine, Tongren etc. (Feng et al., 2006).

Mercury is very toxic to human embryos and fetus, of which methyl mercury plays a major role. It usually exists in various forms such as inorganic, elemental and organic and also it is the only metal which exists in liquid form at room temperature. When mercury has a valence of +2 it is persistently present in the environment (Loux, 1998). Mercury has certain properties such as high volatility and very low level of water solubility. There is a considerable increase in mercury levels seen in the environment where 1500 and 2500 metric tons is said to be released every year (Nriagu, 1989).

A widely used remediation process for mercury contamination is said to be capping and dredging. Dredging helps to obtain a sensible circulation of water, while capping is about placing an appropriate layer of isolating material between the sediments and the water. However an efficient look up is necessary on the type of technique to be used

depending on the contamination level and the surroundings. Though mercury has several forms such as gaseous, particulate etc, in the atmosphere mercury usually occurs in the gaseous form. Major studies demand that atmospheric mercury is the main cause for the contamination in the aquous system (Hermanson, 1993). The contamination of mercury varies from place to place and hence the concentration. Mining, erosion is all contributors of mercury and their function and properties depends on the site they are present on. The remedial methods such as dredging are very expensive. But if there are no high levels of hazards present then the natural attenuation method is highly appropriate (Wang et al., 2004).

Any form of mercury are termed to be poisonous, however alkyl mercury are the ones which have the special properties to break through a biological membrane. It has high volatility and is major risk factor for humans. It is toxic mainly to the central nervous system, and affects human beings through normal activities and also through environment (Crespo-López et al., 2005). It is also shown that small level of exposure towards mercury for a long period shows genotoxicity and sub clinical neuronal abnormal behavior. Mercury can be taken in by clays, oxides, manganese oxides, aluminum oxides and organic matter. However the positive side is that the uptake of mercury from soil into plants is very low (Berzas Nevado et al., 2010).

Blood and hair samples are the usual way of checking mercury levels in humans (Zhang & Wong, 2007). Dentists are said to be constantly exposed to mercury through several forms. However it depends on the level of hygiene maintained at the clinic, safety measures taken and also an appropriate level of ventilation needs to be fixed. However the factors such as age and smoking habits do not influence the level of mercury excreted from the urine (Karahalil et al., 2005). Plants show a certain level of tolerance towards metal pressure. This is either done through avoidance or by increasing its level of resistance. This in turn causes the plant to show increased level of anti oxidants like super oxide dismutase in order to have a better survival. For example plants stressed by mercury showed superoxide dismutase. The genes that resist mercury are termed to be mer operon. The mechanism that is well understood nowadays is the resistance towards inorganic mercury (Osborn et al., 1997). The effects of mercuric chloride are shown to be higher than mercurous chloride. Similarly plants also have varying level of resistance depending on their presence of chromatin and also the availability of sulphur compounds (Patra, et al., 2004).

Mercury has several uses such as insecticides, used in medicine as a purgative and it is also used as rat poison. Asia is the highest in emitting world's largest anthropogenic mercury emission. If 50 percent of emission from Asia can be reduced; it will eventually cause reduction of wet deposition of mercury on a large percentage around the globe

(Schroeder & Munthe, 1998). The regulatory of mercury depends on the toxicity and their effects on their surrounding and the extent by which it can be controlled. Certain regulations are from coal emission which according to EPA's 1999 ICR needs electric utilities for shipment facilities. Certain regulation by EPA and EPRI showed that forty percent of emission was contained at the ashes emitted by coal while the rest of the sixty percent was emitted to the atmosphere. Each and every plant differs in their emission level, of which it varies from ten to ninety percent depending on the level of mercury present in the coal. Though there are several methods in order to control the emission, no best standard method has been implemented or it is not possible to suggest one global method worldwide. Cost of the control methods always play a major role since a standard carbon injection method cost upto 28,000 dollars per lb of mercury eliminated (Pavlish et al., 2003).

This heavy silver metal is being used in barometers, thermometers, lamps, light bulbs, fungicides, batteries, explosives, and paints etc. In spite of its extensive toxicity it is still being used in several applications. However the use of mercury in switches and bulbs are reduced to almost less than fifty percent in countries like America. Gold and silver mining techniques extensively use mercury and hence the formation of contaminated sites. Mercury was always used in separating gold from other sorts of minerals. During the time of precipitation a small amount of mercury is usually washed out. But once it gets converted to an Hg^{2+} form its property changes to dissolve better in water. This is apparently termed to be a wet deposition concept which makes up the reason for most of the arrival of mercury into the surroundings, since only a diminutive amount of particles are emitted to the atmosphere (Gochfeld, 2003).

Oral bacteria are said to contain certain level of resistance towards mercuric chloride. The usage of antibiotics does not affect the flora present in the mouth. However the amount of mercury resistant bacteria is relatively very low. But it was also seen that this Hg resistant bacteria exist more in children than in adults (Ready et al., 2003). Certain bacteria found in south east India were said to be mercury resistant. For example the strains of Bacillus cereus found in the Pulicat Lake had the capacity to lower the water soluble ionic form of mercury into a volatile form. In India about 180 tons of mercury is being released every year into the environment, hence proving that Asia stands the highest in mercury release (Kannan & Krishnamoorthy, 2006).

Certain analysis done quantitatively emphasizes that the soil contaminated by mercury contained the highly resistant microbes. Certain diazotropic bacteria such as pseudomonas and bacillus were seen to be tolerant towards mercury. Mercury takes in the sixth position among the toxic metals worldwide which proves its level of risk posed to the environment. Though soils seem to be similar with respect to texture, color and

composition, the level of these hazardous compounds present in them vary considerably. Most samples are not just victims of one compound rather contain a mixture of other compounds such as arsenic etc. nitrogen fixing bacteria's are quite very sensitive towards heavy metals. However the presence and activity of diazatropic bacteria varies depending on their ability produce and show nitrogenous action. Hence also the most predominant bacteria are said to be proteobacteria and fermicutes showing a good level of resistance. Also in a contaminated soil the micro organisms are termed to be exposed to be metals more prevalently than in an uncontaminated one (Castro et al., 2003). When the number of nitrogen fixators change in a contaminated soil, it eventually shows the environment to be less affected and exists a better functional solidity (Oliveira et al., 2010).

Various strains have been used in order to check the level of metal present in an environment with the help of biosensors. The host that is used extensively is E.coli and every other strain show different levels of response towards metals. For determining the mercury content in the soil, E.coli is not always the effective and environmental host, hence combinations of several biosensor vectors are effective in use. An appropriate sane bacterium towards detection of mercury would be *P. putida* (Hansen & Sørensen, 2000)

Different types of biosensor are also available like bacterial biosensor, plant biosensor where the initial roots and seedlings are grown in the contaminated soil. In order to compare or to determine more effectively the total concentration was identified by AAS method. Any day the biosensor depending on protein and bacteria gives good positive results, whilst the plant biosensor and are not as effective as the former. This toxic metal has relatively high thermal conductivity, has several inimitable properties and this metal's salts are widely used by human beings. One of the advantages of mercury is that some forms of mercury is being used as an ailment for skin and also for eye problems, while metallic mercury has been used in medical applications since many decades. In fact some are also used in minute concentrations as antiseptics (Hobman & Brown, 1997).

The very archetypal method for detection of mercury are AAS (atomic absorption), spectroscopy and inductively coupled plasma mass spectroscopy. However every method has its own drawbacks such as low detection etc. also the main drawback would be that these methods cannot detect the exact characteristic or biological availability of the metal, which is though a metal could be present in an environment it may not be necessarily as toxic as predicted. Hence biosensor plays a good role in sensing at the same time analyzing the metal (Bontidean et al., 2004).

2.3.3 Cadmium

Cadmium is a metal is present naturally in the environment. Its toxicity is usually widespread only through food and the places where cadmium restores itself is said to be kidney. The main problem with respect to cadmium is that it possesses higher risk towards cancer. It was also evident that for people who smoke the level of cadmium in their body was higher. This is because one of the main areas where people are exposed to cadmium is from tobacco. However the higher level always stands through food intake (Olsson et al., 2002). Foods such as crabs, mollusks, oil seeds, cocoa beans, and some types of wild mushrooms all contain cadmium, where especially the kidney and liver of animals are said to contain cadmium on a higher rate. Similarly plants based food products also contain large amounts of cadmium in them. There is always the estimated level that could be allowed for humans for a daily intake and for cadmium its eight to 25 micro grams per day. The intake always varies with respect to the people's diet. People who eat shell fish a lot are prone to cadmium risk quite a lot.

Tobacco intake is vigorously harmful since just one cigarette contains almost 1-2 micro gram of cadmium in it (Willers et al., 2005). On taking note the soils that are contaminated the main reason would be the extensive dust that occurs due to households and certain industries. It's also proved that woman were capable of containing more cadmium in their body than men and this could be because of the fact that woman are capable of more intestinal absorption of their diet than men.

Apart from the dietary aspect and smoking, it is reasonable to say that cadmium are also present in soil, water and air however in air, it's in a relatively low percentage. This intake level seems to be quite high in counties like china and Japan where the intake on estimated level per day seems to be increasing (Joseph, 2009). The way by which cadmium affects humans are mainly through cancer which in turn is caused by a spiteful lump in brain, prostrates, lungs etc. on a growing level it eventually causes loss of function of that area hence affecting the entire function of the body. This attack by a metal develops intensively causing cancer which is done through a special way where the specific site is injured (Templeton & Liu, 2004).

Improper function of DNA could be one of the main major roles in cancer formation, and cadmium which enables a DNA to stop forming its normal function is an added role towards cancer formation. Due to this fact it has a high opportunity leading to gene instability. By means of cell death cadmium attacks the kidney and it is also widely known to form renal cancer. However this risk if formed through an elevated exposure towards pesticides, herbicides, vinyl chloride etc. it was also noted that this was seen in lower levels in females (Hu et al., 2002). Cadmium thereby is said to possess a two type nature or method. One where it enables survival of cells for mutagenesis acting as a

carcinogen and the other as a way of cell death which causes damage of the organ and terminating the function of any particular organ it attacks. Not just the way of exposure and the amount and time of exposure is how cadmium plays its role, rather it enables death also highly depending on the type of cell, and the particular metabolism involved (Templeton & Liu, 2004).

Cadmium's capacity to stay in blood is about three to four months which is termed to be its half life, while if it turns out to be a deliberate component then it could stay up to ten years. If it stays in blood for a really long time with minimal level of exposure, then it occurs as a sign of increased level of cadmium in our body. Even a diet that contains extremely high fiber can be a source for cadmium. Data also shows that 2 percent of cadmium rise in soils are seen every year. In pigs the rise of cadmium level is up to two percent every year while in human kidneys it happens to be much more in an elevated percentage. If a person possess low level of iron, then he is likely to possess more cadmium in blood, which eventually leads to gastro intestinal troubles. Recently it was also studied that a normal excretory level of 2.5 percent in urine could be an indication of renal damage up to about four percent. It is also stressed that if an average uptake of cadmium happens to be 30 micro grams per day then there comes a possibility for about one percent of the individual to obtain cadmium- provoked damage in tubular areas (Järup et al., 1998).

Similar to kidney, reports have also shown that bones are also being affected and stored by cadmium. However kidney is the organ that is being beleaguered more when it comes to aspect such as diet exposure. Cadmium is almost similar to metals such as mercury and zinc and it is also termed that is sometimes dependent on zinc depending on its function. Similarly the amount of urinary cadmium also showed the higher risk of breast cancer, though it is highly related to factors such as age, smoking habits and number of children. Though there was not a large difference seen with cadmium concentration on a normal woman to a woman possessing breast cancer, it was duly noted that human breast tissues contained cadmium binding protein (Antila et al.,1996).

There has been a lot of commission checking the intensity of cadmiums toxicity like the European commission, WHO, IARC, etc. since this metal mostly affects humans mostly though dietary aspect a joint commission of FAO and WHO have taken special interest on checking additives in food and also its respective contaminations (Järup & Åkesson, 2009). Cadmium has the ability to form different type of salts among which sulfate is the most famous one while sulfide posses yellow color pigment. Cadmium is used in batteries and many other such compounds. Proper care needs to be taken for workers handling with cadmium, since any form of cadmium is said to be toxic. Recent interest have emerged onto removing cadmium from waste waters through several treatments such as precipitation, electrochemical treatments etc, however also natural components such as banana pith ,

hazelnut shell and many such types are used in order to reduce cost. In order to remove cadmium pH plays a major role too. Orange wastes are one effective component in order to eliminate cadmium or to absorb them effectively from waste water (Pérez-Marín et al., 2007).

Cadmium is basically a silver-white metal and it is present in the environement through various factors such as volcanic eruptions, burning of coal, through human activity like mining activity etc. it also occurs due to forest fires. This metal since it is extensively found in female's more than male individuals, several scientific survey's were taken which showed evident results that older woman possessed more cadmium levels in their body than younger woman and it was likewise with people who had the habit of smoking. However the woman who had never smoked had the high level possibility to contain the lowest level of cadmium content. Also the creatinine adjusted cadmium levels do not vary extensively on a large basis between individuals. Several factors like age, body weight, diet, children and menopausal stage are always connected with the creatinine adjusted cadmium level in woman though income plays a very minute role and do not contribute towards an increased difference. There are also factors such as multiminerals, vegetables grown in domestic areas and the location of the residence which in no way affects the cadmium level in females. However it's the level of iron in the body that decides the imunity towards cadmium presence in woman. Exposure towards minute level only leads to vomiting and pain in the abdominal area while a high level exposure can cause up to renal tubular dysfunction.

The dysfunction of the renal tube or the problem of proteinuria concerned with cadmium is an effective sign of an improper function in the body. The body burden for people was differentiated with their habit of smoking or non smoking. A good prediction over cadmium intake could be its presence in blood proving its exposure very recently by three months. Few reports also suggest that the intermediary class (Kjellstrom, 1979). Finnish woman have relatively low cadmium levels while woman in the highest class contained a slightly higher level of creatinine adjusted cadmium level in urine. When it comes to men, the level was usually associated depending on their body mass index. Women tend to have higher levels after they pass their menopausal stage. However the people who lived in places where the soil or environment heavily contaminated with cadmium naturally had the problem of higher cadmium level in their body (Sartor et al., 1992).

Though cadmium is quite very well related to zinc, sometimes zinc does have the capacity to reduce the activity of cadmium. Still certain mineral supplements do have the action of cadmium too. It was also seen that woman who took zinc supplements on a continued basis had more cadmium level in their body than those who did not take the

supplements (Kaji et al., 1992). One research was also evidently shown that on a Swedish farming area, the mineral and vitamin supplements users had more of blood cadmium than urine cadmium (Olsson et al., 2002).

Any contamination in the soil based on cadmium will effectively affect the characteristics and the structure of the system in the soil. Certain natural soil organisms are vital for the soil such as some categories of earthworms etc. while contamination of cadmium had shown to create a death rate in these organisms. Several changes arises when cadmium alters the soil structure, such as the level phosphate is reduced, activity of the dehydrogenase is also lowered. However certain components such as beta galactosidose do not get affected. Provided there was also an evident weight loss seen in earthworms which shows that it could be the reason of reduced utilization from the soil due to metal contamination (Ribeiro et al., 2001). Examples of good extractants for cadmium are said to be DTPA, $cacl_2$ solution etc. Adding organic matter will help reduce the effect of cadmium's toxicity in the soil considerably (Tejada, 2009).

2.3.4 Lead

Lead is one of the chemical that is used widely and on a long term basis well identified metal. Though lead has several uses and properties, it still has the capability to cause deleterious effects such as cancer, birth defects, mutation, etc. ever since pre historic time's lead has been used and since then its negative effects has been well known. It is certainly not possible to eliminate it from usage since it has splendid properties and advantages too. During the ancient times lead was being used as an additive in gasoline. It was slowly neglected from such usage due to its harmful effects towards the environment. Not only that, but lead was also used extensively in bottled water, wine bottles, ceramics etc, which were all slowly eliminated from these materials. This is because lead took one of the top ten positions in toxic metals and hence it was vital enough to be taken away from sources that involved dietary aspects. And most importantly those involved usage by children (Bolger et al., 1996).

On an economic level lead is used extensively. On a large level basis it would the storage batteries that occupy the top position in using lead. The several other uses are pipes, sheet, cable covering, etc. there are also several other uses such as for domestic use, like in paints, pesticides, varnishes, hair dyes, astringent, water repellent etc. certain types of lead like lead nitrate are used in matches, explosives, textile printing, chemical reagent textile printing , rodenticide, similarly lead oxide is used in plasters, ointments, iron or steel , varnishes, coloring rubber, matches, etc. lead is also present in quantitative amounts in the environment. Like for example in the north Atlantic and the costal water it quite casually is above 50 ngl^{-1}. It has several reasons to persist in the environment however by a natural cause lead to reach the environment is very meager. For instance, on the northern pacific

regions lead is spread by the environment. In fact this is said to be higher than the emission obtained from lead gasoline. On a comparison rate the amount of lead present in the north pacific seems to be higher than those from the north Atlantic and this can be because of higher or lower pollution by industries (Schaule & Patterson, 1981).

Lead has its elaborate use in so many categories and fields that elimination is of less outlook while reduction is the only explanation. This is because it is also used extensively in cosmetics, applications with respect to medicine, and also in paint from quite ancient times. The adverse effects of lead were analyzed from historic times and it has the ability to make nervous disorder especially in children (Needleman, 1998). Many countries are working towards eliminating lead as much as possible, like United States banned lead from gasoline, while Australia banned lead from paints. This way minor change are being conducted in order to stop future adverse effects. Leads toxicity acts in a way that it deposits itself in several parts of the body. When it comes to adults it stores in bones and teeth while for children it's mostly the bone area. The most suprising effect is that lead has the ability to store itseld in bones for about several years. With the help of erythrocytes it binds itself to it and mediates in the blood and every other sot parts like liver, heart, spleen, lungs, brain etc. the worst criterion is that it has the ability to attack or cause harmful affect to any part in the body. The main mechanism or its special characteristic is that it imitates the deed of calcium and converses itself to protein. (Desilva, 1981). The lead present in blood leads to deleterious effects since its aim is to mostly affect organs such as brain, and kidney. Than adults children are said to be more prone to the risks with lead. Certain factors associated with genes and also with environment can cause neurotoxical problem in children. An increased level of lead found in blood of children has eventually lead scientists to promote educating parents on its adverse effects and its apparent reduction of lead usage (Lidsky & Schneider, 2003).

A study on the lead concentration with pregnant woman is an extensive and an elaborate one conducted by various scientists. Since most of the lead say ninety percent are being deposited in bone and this can certainly affect the fetus when there takes place a maternal bone movement consisting of lead (Schroeder & Tipton, 1968). There are strong evidences that the opportunity for lead to mobilize through blood stream from the mother to the baby by the umbilical cord is quite certain. This could also be due to the reason that more than fifty percentage of woman seem to consume the diet that is less in zinc, vitamin D, iron and calcium. This leads to higher risk in problems associated with lead due to low level immunity and apparent increase in body burden (Schell et al., 2003).

Similarly countries like India are also taking steps in reducing the usage of lead at various places. Once there occurred a non lead petrol period, the concentration of lead in the Ganga River showed a positive decrease. On a more affirmative note the blood lead

levels in children from cities such as Mumbai, Lucknow have also reduced extensively. This has shown so much good signs by which a deliberate decrease in lead levels in the environment is seen causing less adverse effects for the people and environment (Singh & Singh, 2006). The important bases for lead contamination are batteries when recycled, paints, gasoline, several electronic items, and the ancient or conventional medicinal methods used for treatments. Though these methods are quite very effective the ingredients are obtained from barks of trees which are not effectively metabolized by humans due to its hidden metal concentration that is not evident during treatments (Muzi et al., 2005).

A few suggestions and ideas are being made in the prevention, reduction and elimination of lead. Using lead in petrol's are slowly being stopped while, reducing the amount of lead in cosmetics and paints are one effective idea. It is vital to look through the cosmetic ingredient since it has the ability cause cancer. A very effective idea is the chelation treatment, which can help eliminate heavy metals. However not all counties and areas posses the chelating drug and yet another option could be the medical treatment, the tests to identify and eliminate the burden in the body. The main victims of this metal would be the children living in urban areas and the poor ones who are more prone to lead leading to mutilation. Tests were conducted and it showed that not all chelating agent can be effective and it highly depends on the level of lead present and affecting the body condition of the children, however the succimer treatment was also as good as the chelation and yet preventive measures needs to be taken to prevent exposure towards high levels of lead (Rogan et al., 2001).

Technically there are about hundreds and hundreds of products that possess lead as its ingredient or use it as an additive for the material to be formed. Thereby numerous remedy techniques are being suggested in order to control the amount of lead spread through the environment or used by humans as materials. Method like soil remediation is one effective technique. This is mainly used for lead that occurs through smelting and emitted in the environment. Two more important areas are that the use of products containing lead in high quantities can be avoided. Provided maintaining a good hygiene at homes is also a good positive sign. Countries such as china, and India had shown an elevated level of lead in their paints while countries like Singapore has very low level or absolutely no indication of lead in paints. The main area where the control measure needs to be concentrated is the place of work towards lead since workers are prone more towards lead emission and hence control measures and protective measures are extremely vital.

Though many countries have taken good protective measures, there still exists good amount of lead in soils, and atmosphere by some source or the other. Even if eliminated or reduced from the soil it is still possible to find lead in bones of humans exposed through some cause. Besides all this it's also been found that treating children with chelating agents

have not been proven to be hundred percent effective. Hence that way it's necessary to prevent this metal as much as possible in order to avoid hazardous risks and problems in future (Meyer et al., 2008).

An immediate effect or symptom of lead poisoning or body affected by its risk would be depression, coma, death, muscle weakness or cramps. It is also seen that united stated has a rare term effect with lead poisoning while many other countries undergo poisoning of lead frequently while Taiwan is quite well affected by this problem. Primarily it is carbon monoxide problem that affects people while lead is also equally dangerous and affects people with its harmful effects (Liou, 1994).

With respect to carcinogenic effect, its lead acetate and lead phosphate which holds the top position. In fact several studies also explains that exposure towards lead form of salts can lead to cancer problems. Plants and animals have certain immunity or resistance towards toxic metals and for plants it would be phytochelatin which helps itself from protection against metals, also at decisive times. Animals on the contrary possess, methylated to protect themselves from these strong metals. Quite effectively metalathions are proteins which are small in size and highly effective in binding to toxic metals. They also consist of a special characteristic where it can multiply itself into several copies at critical situations. This not only acts effectively towards lead but also towards cadmium, zinc etc. this efficient ion is present not just in plasma, but also in urine, bile etc. micro organisms, plants, animals all have different amount of resistance towards metals. The detailed concept behind how lead causes cancer is still not known however, it has become a source where it is extensively present above natural levels. It is not an intense mutagen and yet it certainly has ability such as causing problems in muscular, intestinal and other vital organ functions. The interest upon lead's action, function and mechanism are still being studied with developing interest in humans due to both its uses and its causes (Johnson, 1998).

A good and reliable method to eliminate lead during acute toxic levels is yet to be found. In any case the primary techniques available are not so effective. Also the present treatment which is the chelating agent only tries to make the lead concentration disappear by means of excreting them. Researchers also insist that nutrition can highly help in fighting against toxic metals.

2.4 Antibiotics

According to Waksman et al., concept of antibiotics stated that 'A chemical substance of microbial origin, that processes antibiotic powers'. However his opinion changed in the year 1961 and the concept of antibiotics has evolved over the time to include plant and animal products as well as synthetic and semi synthetic compounds used in therapy (Yim et al., 2006). In simple words, small molecules that are produced in nature by bacteria and fungi in sufficient quantities to suppress or kill the other micro-organisms could be possibly defined an antibiotic. We can assume that all small molecule made by microbes have biological function. However, some of their environmental activities are not yet known (Davies, 2006).

In the case of infectious disease, biological response of the host and the target microbe elicited by the use of antibiotics may be advantageous and may be detrimental for the outcome of interaction. The expression of antibiotics inside a gene is very important and their response to different species is important too. The pattern exhibited by antibiotics at higher concentration and lower concentration vary and this phenomenon is called as hormesis and this clearly shows the evidence that there is transcriptional change in the host and the pathogen.

The most important outcome is the demonstration that all bioactive small molecules exhibit hormesis; that is, the different phenotypic responses are dependent on the concentration of the bioactive molecule. The primary activity of the most inhibitors of bacterial function is to modulate.

2.4.1 Ciprofloxacin

Ciprofloxacin is a quinolone carboxylic acid derivative and is commonly used in medicine. Ciprofloxacin is a fluoroquinone which is widely used in the antimicrobial therapy. The uses of ciprofloxacin's have been in demand for the past two decades. In pharmaceutical industries the demand for ciprofloxacin has improved considerably. For all the types of ciprofloxacin's, its therapeutic activity is known by the inhibition of an enzyme gyrase (Herbold et al., 2001). Ciprofloxacins are used for oral treatment of cancer and many other diseases like treatment of bone marrow transplantation (van Kraaij et al., 1998). The fluoroquinolones exhibit concentration-dependent bactericidal activity and exert their activity by binding to bacterial topoisomerases II (DNA gyrase). It has been reported that quinolones have some toxic effects on the central nervous, cardiovascular and gastrointestinal systems, and that they also lead to ondrotoxicity, reproductive and developmental toxicity, genotoxicity, carcinogenicity and phototoxicity. The chemical structure of ciprofloxacin is given below in the figure.

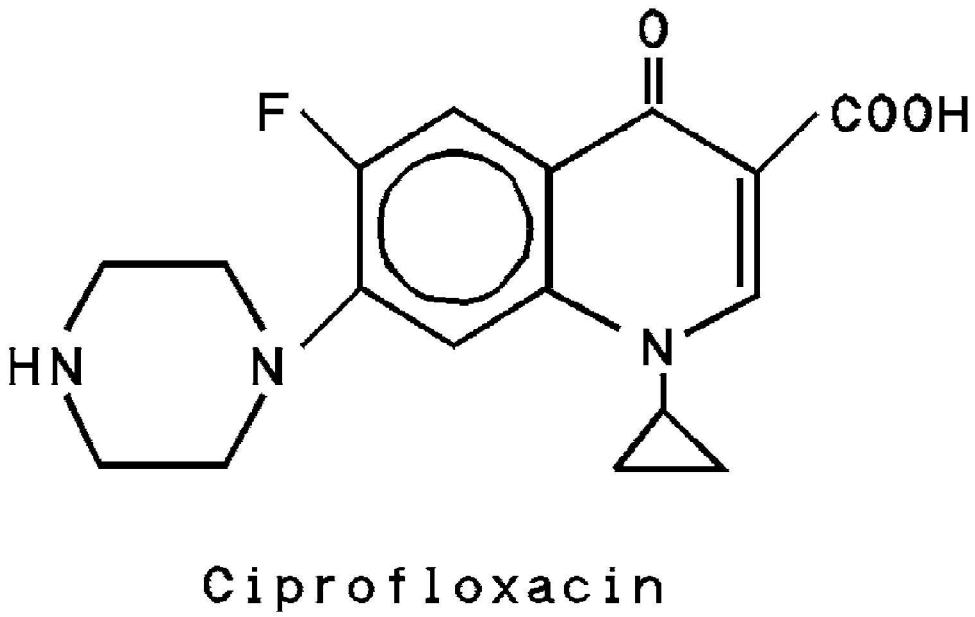

Figure 6 Chemical structure of ciprofloxacin (Herbold et al., 2001)

Nevertheless, there has been a great debate about the reaction of ciprofloxacin towards some resistant bacteria types. Ciprofloxacin being a fluoroquinone can decolonize the bacteria, this not allowing any colony formation of bacteria. Fluoroquinone also inhibits the functionally related mammalian topoisomerase. Apart from all this, ciprofloxacin's have also showed genotoxic effects on gram negative and positive bacteria. However, the antimicrobial activity of fluoroquinones has to be evealuated because; based on the enzyme inhibition and the mutagenic effects, there are possibilities that there can be damage in the DNA. This drug possesses interesting biological properties and potent microcidal activity against urinary tract infection. CFX inhibit DNA replication, repair, transcription, and other cellular functions which cause rapid death of bacteria. CFX also produced immunomodulatory effect on monocytes and macrophages (Dalhoff & Shalit, 2003).

As the studies about ciprofloxacin keeps increasing, there are many researchers and scientist pointing out the ill effects of ciprofloxacin. The toxicological and most dangerous effect of ciprofloxacin has been studied all over the world. The most important fact about ciprofloxacin is resistance to certain bacterial species inside the human colon. And another fact being the mutagenic and the DNA damage caused to a particular strain. Ciprofloxacin is an antibiotic which is widely used in the field of medicine nowadays and side by side there are hundreds of experiments are going on to evaluate and estimate the result about the pros and cons about ciprofloxacin.

Table 2 Comparative activities of ciprofloxacin and other antibiotics (Chin & Neu, 1984)

Organism (no.)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Acinetobacter anitratus (19)	Ciprofloxacin	0.005–6.3	0.4	1.6
	Norfloxacin	0.1–12.5	1.6	6.3
Aeromonas hydrophila (5)	Ciprofloxacin	≤0.002–0.02	≥0.002	0.02
Bacteroides fragilis (23)	Ciprofloxacin	≤0.01–0.8	0.8	0.8
	Norfloxacin	6.3–≥100	6.3	50
Other *Bacteroides* spp. (12)	Ciprofloxacin	0.1–0.8	0.8	0.8
	Norfloxacin	6.3–>100	6.3	25
Branhamella catarrhalis (10)	Ciprofloxacin	<0.01–0.05	0.01	0.05
	Ampicillin	>25	25	
Citrobacter diversus (25)	Ciprofloxacin	≤0.02–0.05	0.01	0.02
	Norfloxacin	0.02–0.2	0.1	0.2
	Cefotaxime	≤0.1	≤0.1	≤0.1
	Ceftazidime	≤0.1–0.2	0.1	0.1
	Cephalexin	3.1–≥100	3.1	50
	Amoxicillin	≥100	≥100	≥100
Citrobacter freundii (22)	Ciprofloxacin	≤0.002–0.2	0.01	0.1
	Norfloxacin	0.02–0.4	0.1	0.4
	Cefotaxime	0.1–12.5	0.2	3.1
	Ceftazidime	0.1–12.5	0.4	3.1
	Moxalactam	0.1–12.5	0.4	3.1
	Amoxicillin	≥100	≥100	≥100
Enterobacter aerogenes (17)	Ciprofloxacin	0.005–0.2	0.01	0.05
	Norfloxacin	0.1–0.8	0.2	0.4
	Cefotaxime	≤0.1–6.3	0.2	6.3
	Ceftazidime	≤0.1–3.1	0.4	3.1
	Moxalactam	0.05–50	0.1	6.3
	Amoxicillin	12.5–≥100	≥100	≥100
	Trimethoprim	0.8–25	3.1	12.5
Enterobacter cloacae (28)	Ciprofloxacin	0.005–0.4	0.01	0.05
	Norfloxacin	0.02–1.6	0.2	0.4
	Cefotaxime	≤0.1–12.5	0.2	12.5
	Ceftazidime	≤0.1–12.5	0.4	12.5
	Moxalactam	≤0.1–12.5	0.1	6.3
Escherichia coli (40)	Ciprofloxacin	≤0.01–0.2	≤0.01	0.02
	Norfloxacin	0.05–0.8	0.1	0.2
	Cefotaxime	≤0.1–0.8	≤0.1	0.1
	Ceftazidime	≤0.1–0.4	0.1	0.2
	Cephalexin	6.3–≥100	6.3	25
	Moxalactam	≤0.1–0.4	≤0.1	0.2
	Amoxicillin	1.6–≥100	≥100	≥100
	Trimethoprim	0.2–3.1	0.2	1.6
Haemophilus influenzae (10)	Ciprofloxacin	≤0.01	≤0.01	≤0.01
	Cefotaxime	≤0.1	≤0.1	≤0.1
	Cephalexin	≤0.1–≥100	12.5	50
	Amoxicillin	≤0.1–25	0.4	25
Klebsiella oxytoca (25)	Ciprofloxacin	0.01–0.05	0.01	0.05
	Norfloxacin	0.05–0.4	0.1	0.4
	Cefotaxime	≤0.1–1.6	<0.1	0.2
	Cephalexin	12.5–100	12.5	100
	Amoxicillin	50–≥100	100	≥100
Klebsiella pneumoniae (29)	Ciprofloxacin	0.005–0.1	0.02	0.05
	Norfloxacin	0.1–0.8	0.2	0.4
	Cefotaxime	≤0.1–1.6	0.1	0.2
	Ceftazidime	0.1–1.6	0.2	0.2
	Cephalexin	0.8–100	3.1	25
	Moxalactam	≤0.1–1.6	0.1	0.2
	Trimethoprim	0.8–≥100	1.6	3.1

Comparative activities of ciprofloxacin and other antibiotics are mentioned in the Table 2. Comparative studies with ciprofloxacin have introduced many possibilities of finding new solution in medicine.

2.4.2 Tetracycline

Tetracycline Antibiotics are intensively used in therapy and many prophylactic control of bacterial infections in human and veterinary medicine (Korpela & Karp, 1998). Tetracycline system is a suitable for studying the antibiotic resistance. Tetracycline binds with the bacterial ribosome and exerts antibacterial action. Tetracycline is very popular and highly known for its bacterial activity and its recommendations on the substances all over the world. Tetracycline is highly used in pharmaceutical industry and in other industries for over three decades. Tetracycline is broad spectrum agents exhibiting activity against gram positive and gram negative bacteria. The advanced antimicrobial property and minimal side effects make it easy and applicable for humans and other animals (Chopra & Roberts, 2001). Thirty-five different tetracycline resistance (*tet*) genesand three oxytetracycline resistant (*otr*) genes have been characterized so far and they have been tabulated in the article by (Chopra & Roberts, 2001)

Mechanism of resistance for characterized *tet* and *otr* genes

Efflux (23)	Ribosomal protection (11)	Enzymatic (3)	Unknown[a]
tet(A), *tet*(B), *tet*(C), *tet*(D), *tet*(E)	*tet*(M), *tet*(O), *tet*(S), *tet*(W), *tet*(32),	*tet*(X)	*tet*(U)
tet(G), *tet*(H), *tet* (J), *tet*(V), *tet*(Y)	*tet*(Q), *tet* (T), *tet*(36)	*tet*(34)	
tet(Z), *tet*(30), *tet*(31), *tet*(33)	*otr*(A), *tetB*(P)[b], *tet*	*tet*(37)	
tet(35)[d]			
tet(39)			
tet(K), *tet*(L), *tet*(38)			
tetA(P)			
otr(B), *otr*(C)			
tcr3			

Figure 7 Showing the mechanism for characterized tet and otr genes (Chopra & Roberts, 2001)

These tetracyclines are absorbed from the stomach and upper intestinal tract. The absorption of the acid is passive. Tetracyclines are widely used in different parts of the world for different applications, most of it being medicinal and there are also other non antibiotic properties of tetracycline that are studied all over the world.

2.4.3 Penicillin

Penicillin is usually produced by a several variety of microorganisms. Many types of techniques such as mutagenic and latest genetic engineering techniques are widely used. Production of penicillin through cell dry weight is considerably very high (Shewale & Sudhakaran, 1997). Penicillin is considered to be one of the most important drugs of the 20th century and is obtained industrially from *Penicillium chyrsogeneum*. In order to improve penicillin in several other aspects, developments are being made in many areas such as genetics, penicillin cell biology etc. There are very many classic tools available and developed in order to study and understand the function and characteristics of penicillin in a better and enhanced way.

Sequencing helps to identify the appropriate and several genes which are available to produce penicillin (Van Den Berg et al., 2008). pH plays an important role in the production of ampicillin with the help of penicillin which is also one of the families of penicillin. When that is done it effectively reduces the adverse reaction or problem of low yield. Penicillin use facilitates several factors from antibiotics up to supporting other effective medicines. This helps reduce the toxicity effect or any side reactions enabling more of a good outcome from a reaction (Ospina et al., 1996) .

Notably penicillin has different forms such as amoxyllin, penicillin acylase, ampicillin etc. however it undergoes an initial conversion to 6 amino penicillinic acids, in order to form the above forms for an industrial or enzymatic use. The most famous beta lactam antibiotics are penicillin since they have a high level effectiveness in attacking the cell walls of bacteria. It has a very effective anti bacterial activity, and above all most importantly considerably low level of toxicity or side reactions. However any antibiotic when used in excess amounts will eventually lead to resistance by the microbes at some particular point. This problem of resistance by bacteria could be trounced by forming semi synthetic penicillin's. There are certain methods in order to form these conversions yet chemical methods are always found to be effective with relatively cheap rate yet they also possess the burden of using heavy and risky chemicals like pyridine nitrosyl chloride etc. on the positive side the way in which the transformations are made or the condition in which it is formed is said to be placid.

Large amounts of penicillin are being produced every year and eventually on the year of 2000 penicillin production has risen to about seven thousand tons. Several aspects are needed to be considered and if a cheap enzyme catalyst is found then the factors such as rate, activity , stability etc can enable to lower the cost of the process in a very effective way thereby creating the semi synthetic penicillin needed- 6- amino penicillinic acid. Microbes are also yet another major contribution towards formation of penicillin products.

The main kind sof microbes that are involved are E.coli, proteus retgerri, *penicillium chrysogenuem* etc, *achromobactor sp*, the details regarding their structure and their respective molecular weights are also an important aspect with respect to production which have all been tested for an effective production (Sudhakaran et al., 1992).

Similar processes are performed for penicillin with respect to purification as how steps are involved with enzymes or byproducts. Penicillin also has its own purification methods, and the most specific ones consist of fermentation broths, filtration and also methods such as precipitation and homogenization methods. A good purification needs to involve breakage of cells and to remove the unwanted cell wastes, and impurities. Very much importance is given to the part of disrupting the cells, due to its elevated importance that is gained over the years and also due to its vital necessary in the process. Though a process that is primary, efforts are made to improve and make it to a larger process scale conditions in an advanced way (Chisti & Moo-Young, 1986).

This is all eventually followed by elution and apparently gel filtration chromatography. Though these are the general outline, when it happens to be a commercial product it's more concentrated on the commercial level with less number of steps involved. When it comes to industrial aspect, stability is very important. Several alterations such as elevating the solubility level of reactants and lowering the reaction medium's viscosity are all important. If the percentage of free water is well lowered then the mobility can be reduced too. While for such an action to take place stabilizing agents need to be used. For penicillin a good thermo stability can be achieved by using PEG 4000 or 6000. Hence these methods are readily available and very effective towards purification and virtually towards stabilization of penicillin. On an important aspect these methods are quite vital due to its leading position as an antibiotic.

The enzyme is said to be made stable by using cross linking agents despite of any pH conditions. A huge advantage over penicillin is when it is immobilized and commercially immobilized penicillin is said to be very famous since it is very economic and much more useful. This is because it is with more specificity and stability and has considerably less contamination and a good reaction rate. There are also some problems that occur with the immobilized penicillin but that only when there is a multipoint covalent attachment. For penicillin to be a good antibiotic or for it act accordingly with respect to its properties its side chain needs to be appropriate. This takes place only when certain organisms accept and produce these side chains. However any enzyme when mastered or positively changed with mutagenesis works well and basic understanding of its action is always vital towards its entire process (Parmar et al., 2000). The performance of immobilization procedure has only made the enzymatic process more convenient and accessible enough. Provided the yields of these methods are considerably higher than the

old classic method routines (Bruggink et al., 1998). Penicillin also possesses its own side effects when used in excess or by improper medical advice. It can cause diarrhea, fever, vomiting, nausea etc. however it also depends on how one individual hold on to it depending on their body capacity. There is been a high stipulation for penicillin G acylase and annually it is produced in the amount of say 10 to 30 million tones. It is effectively produced in high quantities by using *E.coli* and *bacillius magaterium* organisms. When it comes to commercialized conditions, the circumstance for fermentation could be changed in order to get a better penicillin product.

On a top leading market sales beta lactam antibiotics are said to possess one of the leading positions. The two main things that need to be concentrated are the organism used and the fermentation broths. Hence the vital organism being penicillium chrysogenum and acremonium chrysogenum and an improved technique with fermentation process can effectively reduce the cost factor for the production criteria. But in spite of all these factors, all these also require intensive labor, raw materials needed and also the energy, countries such as Korea and India have started to become a leading producers in production of penicillin while European countries which were leading once are slightly lagging behind (Elander, 2003).

The uses of micro organisms are a great importance and an advantage for the production of penicilin G acylase. By improving the strains, with the help of cloning and expression, good metabolites can be obtained. Similarly mutagenesis also helps in facing changes according to the molecular level. For example site directed mutagenesis always helps in finding out a changed sequence of amino acid, or a cloned gene of penicillin etc. this process will considered very important for any industry performing fermentation with respect to medicine since it's a good opportunity to ascertain its form. Identifying the amino acids, tracking the mutagenesis level with site directed or evolution mutagenesis experiments and determining the experiments specificity helps to explain the accessibly of penicillin on a molecular level (Roa et al., 1994).

If penicillin acts as a hydrolase or transferase then it is quite possible for it to undergo unwanted side reactions. Penicillin and its action along with cephalosporin acts effectively in the formation of 6 amino penicillinic acids and this could be appropriately controlled with the help of certain kinetics or by using biotransformation. A good improvement in a PGA strain can help improve the production of beta lactam antibiotics (Chandel et al., 2008). The notable thing and a special characteristic would be that penicillin is the first antibiotic that was found and even after several many years it is still one of the best antibiotics which is being used in therapeutics and consistently produced in large amounts every year. Initially it was obtained from penicillin notatum but eventually it is gone in to a wider horizon where it is obtained from several other organisms among

which *Penicillium chrysogenuem* is a very important source. It is continuously seen that good effective classic methods have not given much pathway towards new DNA recombinant methods; however findings on new organisms and improvements on fermentation methods can enable to establish a good opportunity for the industries.

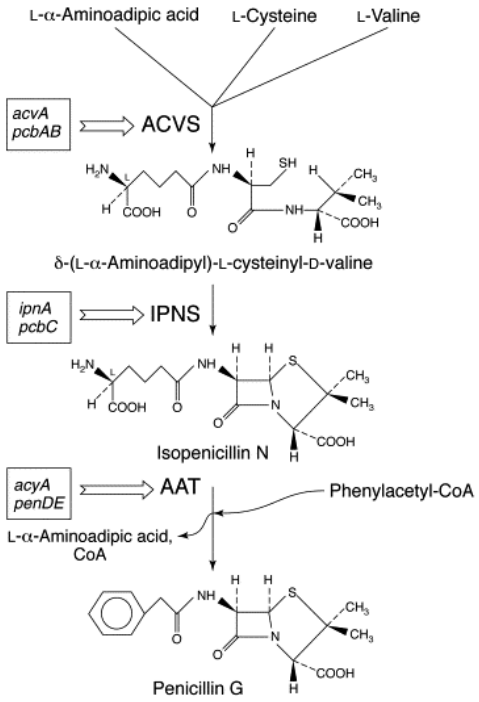

Figure 8 Biosynthsis of Penicillin (Peñalva et al., 1998).

The above picture is the biosynthesis of penicillin G. these steps are followed and performed by three enzyme encoded by one gene. The gene names are boxed while the gene is encoded (Peñalva et al., 1998).

Unlikely on a higher level, sucrose or glucose has the capacity to lower the production of penicillin. Yet industries do not take great interest in these areas. Ever since peniclillin was discovered it's been almost seventy years and since then it has seen a considerable rise in improvement with respect to mutation, technique for selection and one of the highest producers. Many steps are improved with respect to production and still many are needed to be known when it comes to the areas of target, reduction of certain side effects and also based on strains and the energy required for its production.

3. Research methods and Protocols

Chemicals used in the experiment:

Chemicals for the experiments were used from the laboratory of environmental engineering and the chemicals used are mentioned below. Ciprofloxacin (Bayer), Sodium chloride (NaCl) from BDH (England) Yeast extract from Scharlau (Barcelona, Spain), Tryptone BD from (Becton Dickinson and co., USA), Bacto agar from BD, (Becton Dickinson and co., USA), Ampicilin from Sigma (USA) and H_2O Octavia (Norway).

Preparation of stock/samples:

The stock solution (30mg/ml) of **c**iprofloxacin was prepared by dissolving the powdered ciprofloxacin in milliQ water. From the stock solution dilutions were made by adding 250µl of ciprofloxacin and 750µl of milliQ water. Similar dilutions were made in 10 vials and specific concentrations were picked up for experimental usage. The selected concentrations are 7.5, 0.03, 0.002 and 0.0001 mg/ml and they were stored at 4°C. Similarly Arsenate and Arsenite stock solutions were prepared from Arsenite (III) and arsenate (V) standards were prepared from three different arsenic compounds. Stock solutions of 10 mM arsenite $NaAsO_2$ (III) and arsenate Na_2HAsO_4 (V) and As_2O_5 (V) (obtained from Fluka Chemica, Switzerland) were prepared by weighing and diluting each arsenic sample in MilliQ-water at pH 7. Arsenic compounds were of analytical grade.

Calibration standards for arsenite and arsenate solutions were diluted from the concentrated stock solution. Calibration curves were selected from nine arsenic concentrations: 30 µM and 100 µM were used as final Arsenic concentrations. All solutions were prepared with deionized and purified water at pH 7 by Milli-Q Plus system (*Millipore, MA).*

Preparation of Growth Media:

Luria Bertani (LB) broth was prepared by dissolving yeast extract (5 g /liter), tryptone (10 g/liter) and NaCl (5 g/liter) into MQ water and supplemented with or ampicillin (100 µg/ml) after autoclaving at 121°C for 15 min. The same medium was solidified with Bacto agar 7.5 g/liter resulting in Luria Bertani agar (LA) and soft agar, where as hard agar was (15g/liter) respectively. LB was used in bioluminescence assays and in preculture of IVIS agar diffusion assays, while LA and soft agar were mainly media of IVIS agar diffusion assays.

Biosensor strains and growth conditions:

The strains were stored in a 20% glycerol suspension at - 85°C. Initially, the sensor strains were grown on LA plates overnight at 37°C and selected individual colonies were picked for cultivation overnight at 37 ˚C with 300 rpm in LB broth. Before the start of the

bioluminescence measurements,the culture was diluted with the same medium to OD_{600} of 0.2-0.3. Depending on the OD from the overnight pre-culture, the incubation time may vary from 1 to 2 hours for diluted cells. This is done by adding 10 ml of LB in a sterile conical flask supplemented with ampicillin and shake at 37°C and 300 rpm.

3.1 Comparison of Bioluminescence for heavy metals using luminescent counters

3.1.1 Bioluminescence counting using Chameleon

The arsenic biosensor strain used for luminescence measurements was freeze-dried *Escherichia coli* XL-1 (parsRluxCDABE). The sensor cells were freeze-dried to diminish batch-to-batch variation and reagent-like usage of the biosensor without tedious culturing of the cells. Freeze-drying also allows permanent storage of the reporter systems. Rehydration of the freeze-dried cells was done with distilled water. Rehydrated freeze-dried sensor cells were diluted before luminescence measurements in Luria Bertani (LB) medium (10 g of tryptone, 5 g of yeast extract, and 5 g of NaCl per liter, pH 7).

For IVIS agar diffusion assays the arsenic biosensor strain were stored in a 20% glycerol suspension at - 85°C. Before culturing, the sensor strains were grown on LA plates overnight at 37°C and stored as colonies at 4°C until further use. Prior to IVIS agar diffusion assays, the strain was cultivated overnight (preculture) at 37 ˚C with 300 rpm shaking in LB broth. Before the start of the bioluminescence assay procedure the culture was diluted with the same medium to OD600 of 0.2-0.3 indicating the background level of luminescence measured by the Chameleon Multilabel Detection Platform (Hidex Oy, Turku, Finland).

Freeze-dried *E. coli* XL- 1 parsRluxCDABE sensor cells (Hakkila et al., 2004) were rehydrated by adding 1.0 ml of distilled water into an ampule and allowed to stand for 2 hours at room temperature. After rehydration luminescence measurements were done same way as with fresh cells. Reconstituted cells were diluted before assay measurements 1:10 with Luria Bertani medium (LB) to decrease the background luminescence level.

Assay mixtures were prepared directly in the white 96-well microtiter plates (Thermo Labsystems, Helsinki, Finland). First, 100µl of each arsenic calibration standard solution was added to plate. Reconstituted and diluted cell suspension in a volume of 100µl was added to each well just before first measurement. All mixtures were measured as triplicates. Arsenic 30µM both for As (III) and As (V) samples were added on to the plate. After addition of biosensor cells, the plates were shaken briefly (300 rpm) by a plate shaker (Biosan Thermo Shaker, Labema Oy, Kerava, Finland) and luminescence at zero-point was

measured. The 96-well plates with the assay mixtures were covered with sticker and incubated with shaking (300 rpm) at 37 °C until the next measurement time point. During incubation, the luminescence was measured once per hour from the starting point for total of 180 min (0h, 1h, 2h, and 3h) by using the Chameleon Multilabel Detection Platform luminometer (Hidex Oy, Turku, Finland). Induction coefficients were calculated using the formula IF = Li/L_b, where IF is the induction Factor, *Li* is the luminescence value of the sample, and L_b is the luminescence value of a blank noninducing sample using L*i* and L_b from the same time point.

3.1.2 Bioluminescence counting using Wallac Victor[2]

Assay mixtures were prepared directly in the white 96-well microtiter plates (Thermo Labsystems, Helsinki, Finland). First, 100µl of each arsenic calibration standard solution was added to plate. Reconstituted and diluted cell suspension in a volume of 100µl was added to each well just before first measurement. All mixtures were measured as triplicates. Arsenic 30µM both for As (III) and As (V) samples were added to the plates. After addition of biosensor cells, the plates were shaken briefly (300 rpm) by a plate shaker (Biosan Thermo Shaker, Labema Oy, Kerava, Finland) and luminescence at zero-point was measured. The 96-well plates with the assay mixtures were covered with sticker and incubated with shaking (300 rpm) at 37 °C until the next measurement time point. During incubation, the luminescence was measured once per hour from the starting point for total of 180 min (0h, 1h, 2h, and 3h) by using the Victor Wallac Multilabel Detection Platform luminometer (Perkin Elmer life sciences, USA). Induction coefficients were calculated using the formula IF = Li/L_b, where IF is the induction factor, *Li* is the luminescence value of the sample, and L_b is the luminescence value of a blank noninducing sample using L*i* and L_b from the same time point

3.1.3 Bioluminescent counting using Xenogen Imaging station

An agar diffusion assay (ADA) was performed for the comparison of arsenic. Logarithmically grown cells (*E. coli* XL-1 parsRluxCDABE) were added to soft agar supplemented with appropriate antibiotic (ampicillin), IPTG and 0.2% glucose were mixed gently and poured on top of the LA agar plates containing fish filet (Baltic herring, *Clupea harengus membras*) samples soaked in 5 ml of 30 µM arsenic (Fig. 2a) and 5 ml of milliQ water (Fig. 2b) for 24 hours. Biophotonic imaging station (IVIS Xenogen, Caliper Life Sciences, USA) was used to visualize the different arsenic comparison. Exposure time at each measurement point was 30 sec.

Similar experiment was done on a 6 well plate to compare the effectivity of two oxidation states of arsenic one below the other. Logarithmically grown cells (*E. coli* XL-1 parsRluxCDABE) were added to soft agar supplemented with appropriate antibiotic (ampicillin), IPTG and 0.2% glucose were mixed gently and poured on top of the LA agar

plates containing of Arsenite and Arsenate. Pictures were taken for three hours to check the bioluminescence present in the samples.

On the other hand Whatman filter discs (Ø 6mm) containing 20 µl of Arsenite were placed on top of the soft Agar. Four discs with different arsenite concentration were poured to be examined. The highest being 30µm and the lowest being 1µm and milliQ water served as a blank. Readings were taken for three hours.

3.2 Comparison of Bioluminescence for ciprofloxacin using photon counters

3.2.1 Bioluminescence counting using Chameleon

The biosensor strain used for luminescence measurements was freeze-dried *Escherichia coli* XL-1 DPD2794. The sensor cells were freeze-dried to diminish batch-to-batch variation and reagent-like usage of the biosensor without tedious culturing of the cells. Freeze-drying also allows permanent storage of the reporter systems. Rehydration of the freeze-dried cells was done with distilled water. Rehydrated freeze-dried sensor cells were diluted before luminescence measurements in Luria Bertani (LB) medium (10 g of tryptone, 5 g of yeast extract, and 5 g of NaCl per liter, pH 7).

Fifty micro liter aliquots of the sample set were pipetted in triplicate into a white 96-well plate and fifty micro-liters of diluted bacteria pre-culture were then added into the wells containing the samples. The samples were measured with a Chameleon Multi-label Detection Platform (Hidex Oy, Turku, Finland). After addition of biosensor cells, the plates were shaken briefly (300 rpm) by a plate shaker (Biosan Thermo Shaker, Labema Oy, Kerava, Finland) and luminescence at zero-point was measured. The 96-well plates with the assay mixtures were covered with sticker and incubated with shaking (300 rpm) at 37 °C until the next measurement time point. During incubation, the luminescence was measured once per hour from the starting point for total of 180 min (0h, 1h, 2h, 3h, 4h, and 5h) by using the Chameleon Multilabel Detection Platform luminometer (Hidex Oy, Turku, Finland). Induction coefficients were calculated using the formula IF = Li/L_b, where IF is the induction Factor, Li is the luminescence value of the sample, and L_b is the luminescence value of a blank non inducing sample using Li and L_b from the same time point.

3.2.2 Bioluminescence counting using Wallac Victor [2]

Assay mixtures were prepared directly in the white 96-well micro-titer plates (Thermo Labsystems, Helsinki, Finland). Samples were loaded on the plates in a same way as in that for chameleon. Then the plates were measure for 5 hours (1, 2, 3, 4 and 5 hours). were

shaken briefly (300 rpm) by a plate shaker (Biosan Thermo Shaker, Labema Oy, Kerava, Finland) and luminescence at zero-point was measured. The 96-well plates with the assay mixtures were covered with sticker and incubated with shaking (300 rpm) at 37 °C until the next measurement time point. Luminescence measurements were made in Victor^2 wallace multilabel plate reader (Perkin Elmer life sciences, USA) in Induction coefficients were calculated using the formula IF = Li/L_b, where IF is the induction Factor, *Li* is the luminescence value of the sample, and L_b is the luminescence value of a blank non inducing sample using L*i* and L_b from the same time point.

Opaque microtitre plates should be used. Black plates exhibit less "crosstalk" owing to reflection of light from one well into neighboring wells. These are especially useful for strains with high background transcription and large induction potential, such as the DPD 2794 (recA::lux) strain. By reflection whit plates enhance light fluence at the detector from strains that produce lower amounts of light.

3.2.3 Bioluminescence counting using Xenogen Imaging station

An agar diffusion assay (ADA) was performed for the comparison Antibiotic CFX. Logarithmically grown cells *Escherichia coli* XL-1 DPD2794 were added to soft agar supplemented with appropriate antibiotic (ampicillin) and mixed gently and poured on top of the LA agar plates. Whatman filter discs (Ø 6mm) are placed on top of the soft agar. Different concentrations of ciprofloxacin (blank, 0.0001, 0.002, 0.03 and 7.5 mg/ml) were poured on top of the filter discs. Biophotonic imaging station (IVIS Xenogen, Caliper life sciences, USA) was used to visualize the different arsenic comparison. Exposure time at each measurement point was 30 sec. Pictures were taken for five hours to check the bioluminescence present in the samples. Readings were taken with 1 hour intervals for 5 hours. Luminescent zones indicated SOS response. The SOS response was measured as mentioned in (Belkin et al., 1997).

4 Results

4.1 Bioluminescence counting for Arsenic Biosensor

Luminescence values are represented as IF (for particular instruments output). The kinetic profile of *E. coli* XL-1 parsRluxCDABE had a 40-60 minute lag or in other words low luminescence was seen in the first one hour. Based on the studies from (Hakkila et al., 2002) it is known that the bacterial strain is dose dependent. However, same concentrations of As (III) and As (V) samples were used.

4.1.1 Bioluminescence counting using Chameleon

Oxidative stress plays an important role in the measurement of arsenic induced toxicity (Shi, Shi, & Liu, 2004). As mentioned by Shi et al., 2004 and others the oxidative stress of arsenic could be measured by many methods (Hakkila et al., 2002). Experiment conducted in real time assay results are worked on a spreadsheet. The intensity of the bioluminescence is proportional to the arsenite concentration in the luminescence assay measurements; detection limit being 0,3 µM. Higher the concentration of Arsenite and Arsenate, higher the luminescence produced

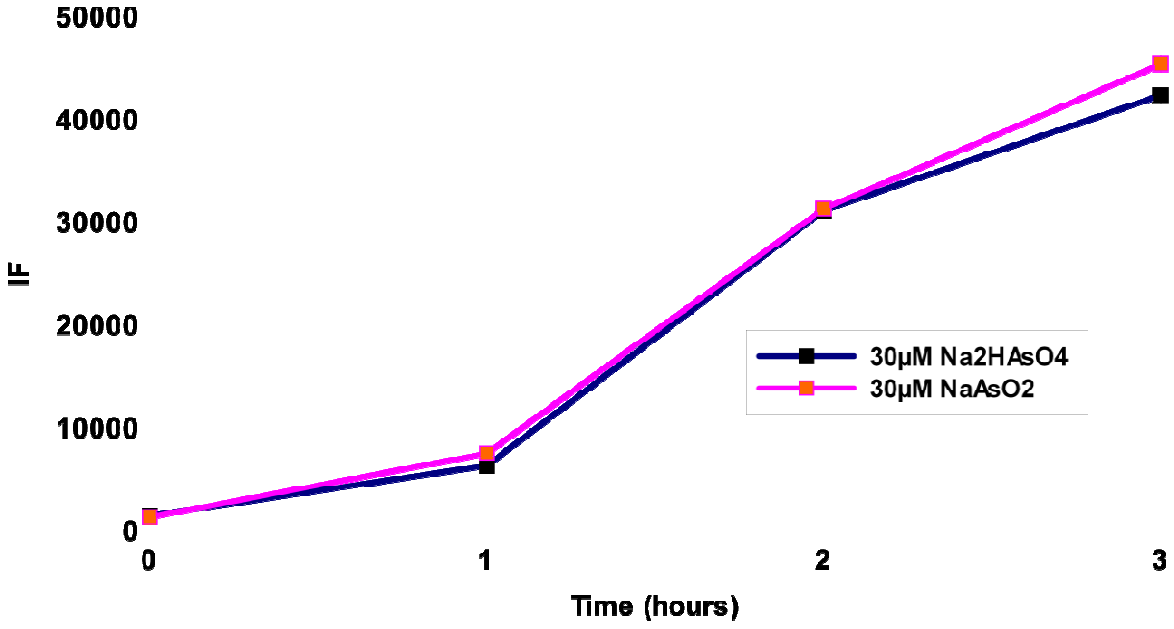

Figure 9 *Luminescence counting using chameleon. Measurements were made for three hours and the graph was plotted for 30µM Na2HAsO4 and NaAsO2 exposed to E. coli XL-1(parsRluxCDABE) for 3 hours.*

The induction factor for Arsenite (III) and arsenate (V) was calculated. Figure 9 shows the schematic real time assay of *E. coli* XL-1 parsRluxCDABE, an arsenic sensor. After the addition of sensor cells and 30µg/ml As (III) and As (V) samples kinetic real-time curves were drawn for each sample measured.

4.1.2 Bioluminescence counting using Wallac Victor[2]

The advantage of biosensor is high compared to physical and chemical analysis. Hence this arsenic based biosensor will serve as a tool for detection of arsenite and arsenate (Yagi, 2007). Hakila et al constructed the Arsenite sensor with lux genes and detected the limit and linearity (Hakkila et al., 2002). The Analysis of the data obtained is done using a spreadsheet (e.g., Excel or Gnumeric). It is helpful since the luminometer had an interface physically linked to a PC.

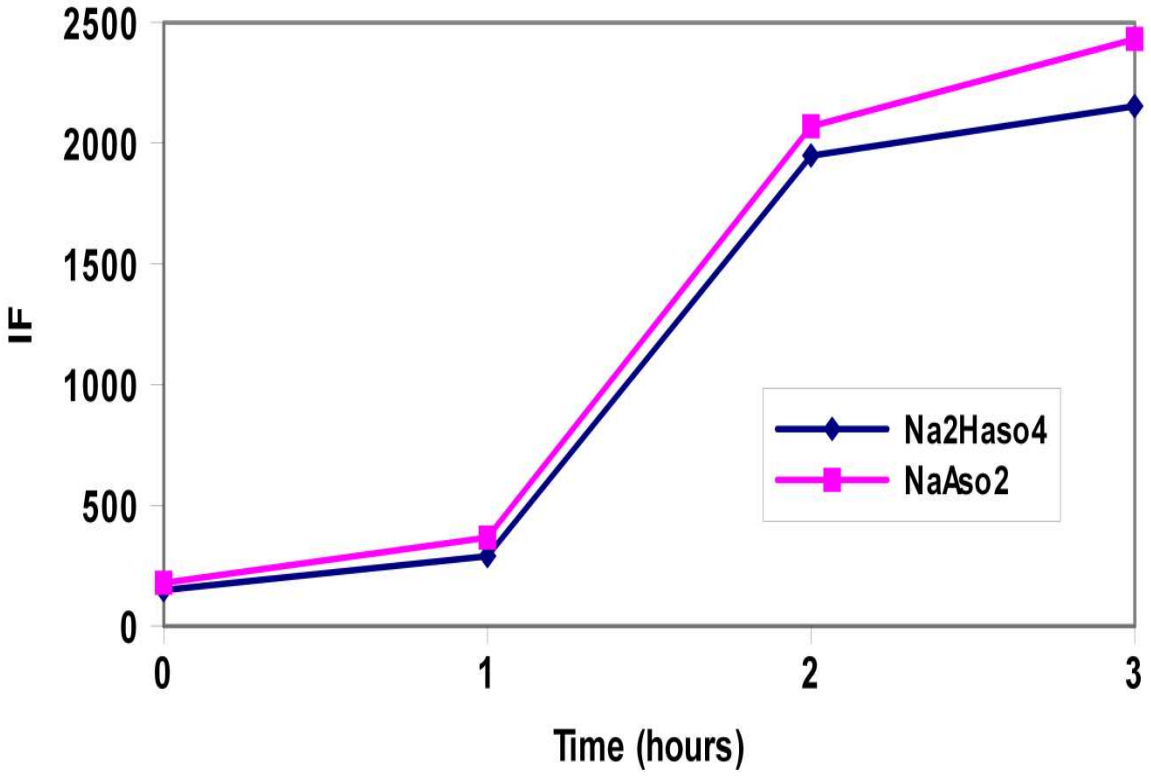

Figure 10 *Luminescence counting using Wallac. Measurements were made for three hours and the graph was plotted for 30µM Na2HAsO4 and NaAsO2 exposed to E. coli XL-1(parsRluxCDABE) for 3 hours.*

The degree of luminescence produced is expressed in terms of IF (induction factor). IF = Li/L_b, where IC is the induction coefficient, *Li* is the luminescence value of the sample, and L_b is the luminescence value of a blank non inducing sample using L*i* and L_b from the same time point.

Figure 9 shows the schematic real time assay of *E. coli* XL-1 parsRluxCDABE, an arsenic sensor. After the addition of sensor cells and 30µg/ml As (III) and As (V) samples kinetic real-time curves were drawn for each sample measured. The induction factor of arsenite (2400) is found to be higher than the induction factor of arsenate (2100).

4.1.3 Bioluminescent counting using Xenogen Imaging Station

Baltic Herring soaked on different concentrations of Arsenite (III) and arsenate (V) was luminescent photographed in (Xenogen VIVO Vision IVIS Lumina, Caliper Life Science System, USA) for 3 hours. Higher optical density resulted in early saturation of the image. The image was Post-processed using Living image 3.1 software. The transition of colour of the luminescent image was evident for the theoretical fact. The regions around the 24 hour soaked *Baltic herring* fish sample were starting to get more luminescent and there was scattering of the arsenite and arsenate. Figure11 shows the luminescent image of Baltic Herring fish samples in arsenic biosensor

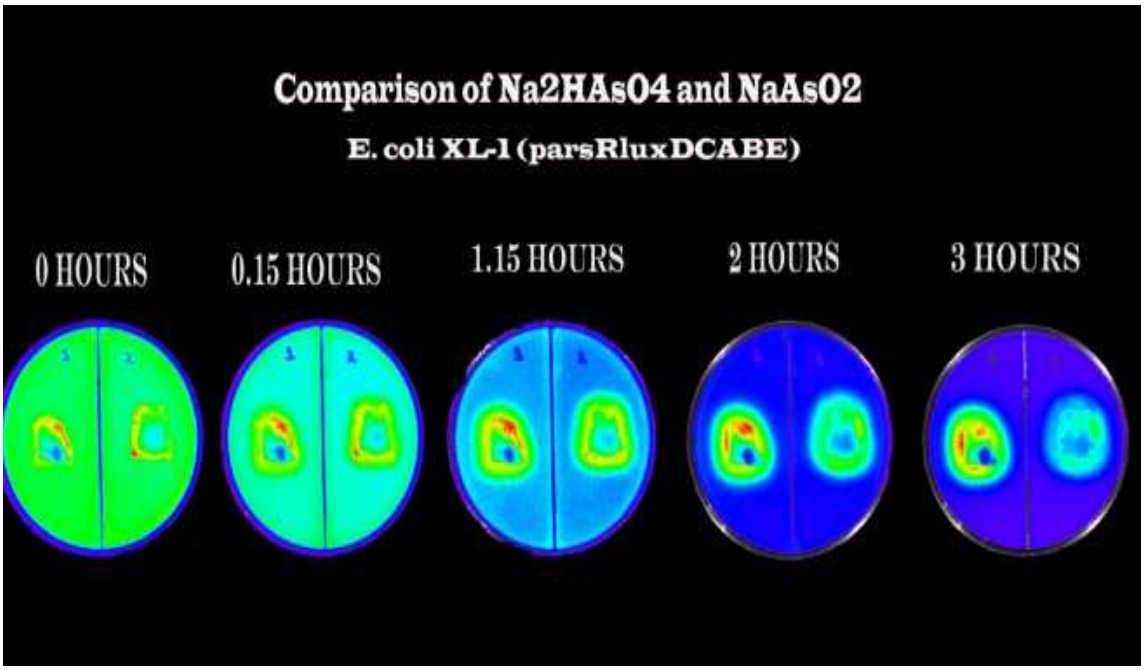

Fig.11. *Comparison of arsenic Using fish sample immersed in 5 ml of 30µM Na2HAsO4 and NaAsO2 for 24 hours and exposed to E. coli XL-1(parsRluxCDABE) for 3 hours*

The luminescent overlay photograph at time 0 hours showed the overall region of the plate to be luminescent with light green colour. Light green means, luminescent data lies in the moderate photon region. However, the luminescent region did not remain the same as time increased. There was reaction between Arsenate/Arsenite and *E. coli XL-1(parsRluxCDABE)*.

Analysis of the data obtained is done using a spreadsheet (e.g., Excel or Gnumeric). The data obtained from fish fillets were saved on a spreadsheet and the resulting graph was modified to compare the luminescence with the samples obtained for three hours.

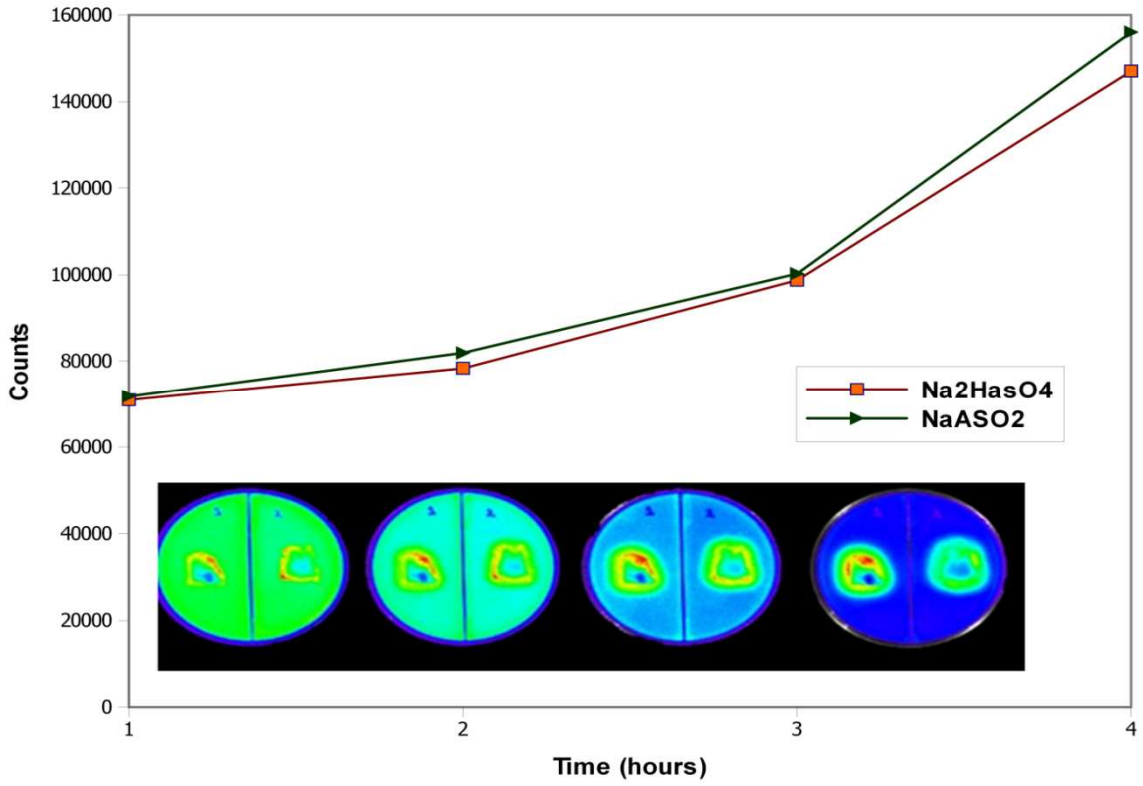

Figure 12 *graph of arsenic Using fish sample immersed in 5 ml of 30μM Na2HAsO4 and NaAsO2 for 24 hours and exposed to E. coli XL-1(parsRluxCDABE) for 3 hours.*

Figure 12 represents the Increase in counts of the arsenic forms, how they differ in width and which oxidative form of arsenic acts more vibrantly compared to the other.

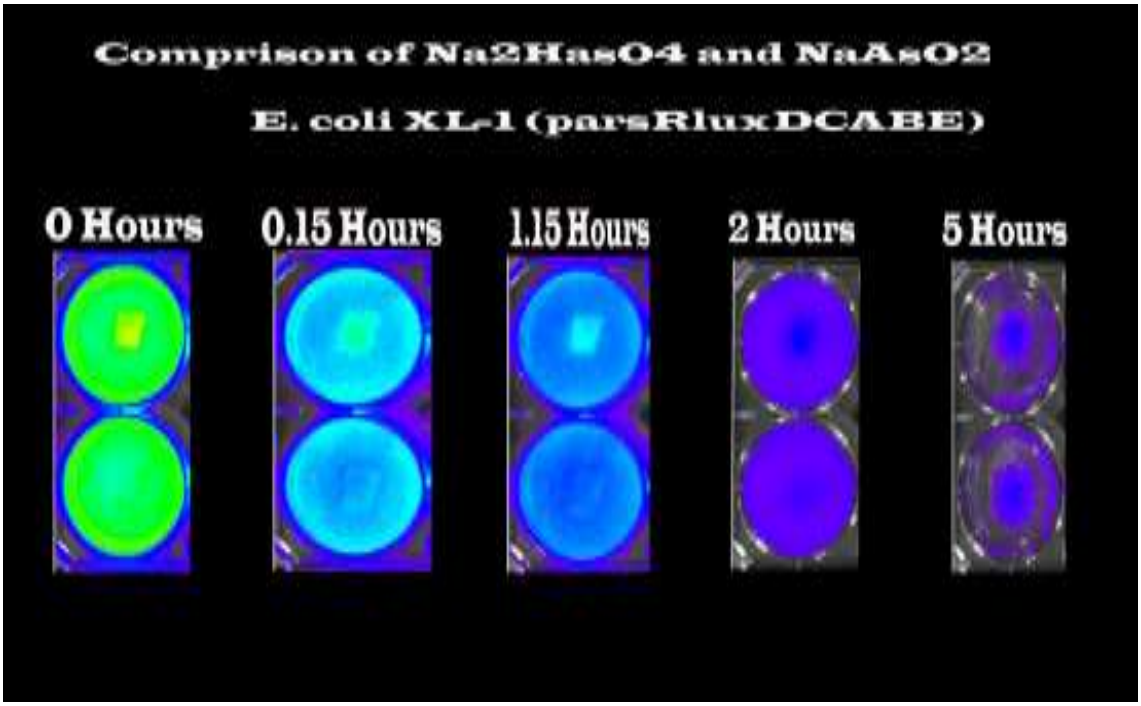

Fig.13 F*ish sample immersed in 5 ml of milli-Q water for 24 hours and exposed to E. coli XL-1(parsRluxCDABE) for 3 hours.*

In order to confirm the activity of arsenite (III) and arsenate (V) on *E. coli* XL-1 parsRluxCDABE a 6 well plate was used to determine the differences by immersing Baltic herring in Mill Q water for 24 hours. There was no exorbitant luminescence in the fish sample, which indicated that arsenic induced luminescence in the fish samples in fig (13). However, the region around the fish fillet started to decrease

4.1.4 Visualization of Bioluminescent bacteria using circular discs

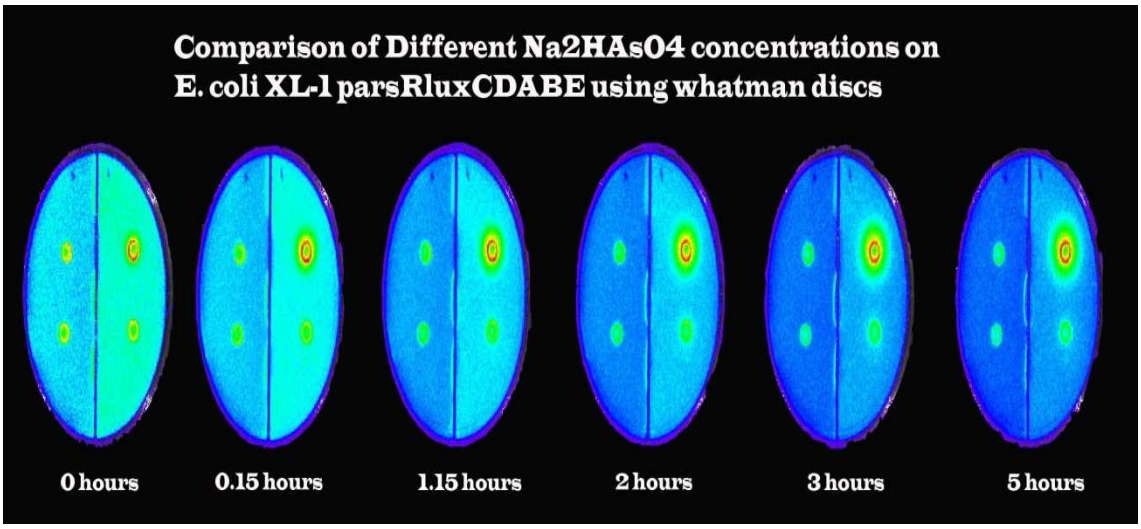

Figure 14 *Picture taken in laboratory of Environmental engineering and biotechnology (1-12-09) using IVIS Lumina Xengonen. Picture shows the comparison of different concentrations (blank, 1 µm, 3 µm, 30 µm) of Na2HAso4 for 5 hours.*

Procedure mentioned above in 4.3.1 was performed .Four filter discs were placed on the agar plates with differencing concentrations. Initially all four discs in the Petri dish looked almost similar and after1.15 hours, there was a prominent increase in circular disc containing 30µm concentration. Figure 14 shows luminescence on disc containing 30µm of Arsenite kept increasing dynamically with time, whereas the circular disc with 3µm increased only very little. There was no increase of luminescence in the blank and almost no increase of luminescence in 1µm concentration. Potential light emission from the particular strain is noted and the dose response assessment was noted. Higher the dosage of the arsenite and arsenate resulted in greater luminescence on the agar plate.

Hence the objective of comparing the results obtained from circular disc to one with the fish is positive. The reaction with Baltic herring showed the similar reaction when exposed to higher arsenite and arsenate concentrations. Similar results were seen in filter discs.

4.2 Comparison of Bioluminescence for Antibiotic, ciprofloxacin using luminescent counters

DPD2794 is also known as DNA damage sensor, it responds quickly for the presence of genotoxicants (Belkin et al., 1997).Ciprofloxacin a fluoroquinone (Herbold et al., 2001) is used for the estimation of efficiency of luminometers. Bioassays were aimed at gathering potential light emission from the particular strain responding in a particular pattern for each strain. The luminescence emitted by DPD2794 was considered to be 10fold higher compared to strains of the same category.(Belkin et al., 1997)

4.2.1 Bioluminescence counting using Chameleon

Luminescence readings were carried out from 0-5 hours. Luminescent kinetics shown below in figure 15 shows the presence of different ciprofloxacin concentrations as proved by Bechor et al. Two humps seen in the graph, represents the behavior of Ciprofloxacin on DPD2794. However, single test cannot provide a definite and comprehensive picture of the whole phenomenon. Readings were plotted as time in X axis and Counts in Y axis. The noise reduction or the background level was subtracted on a worksheet.

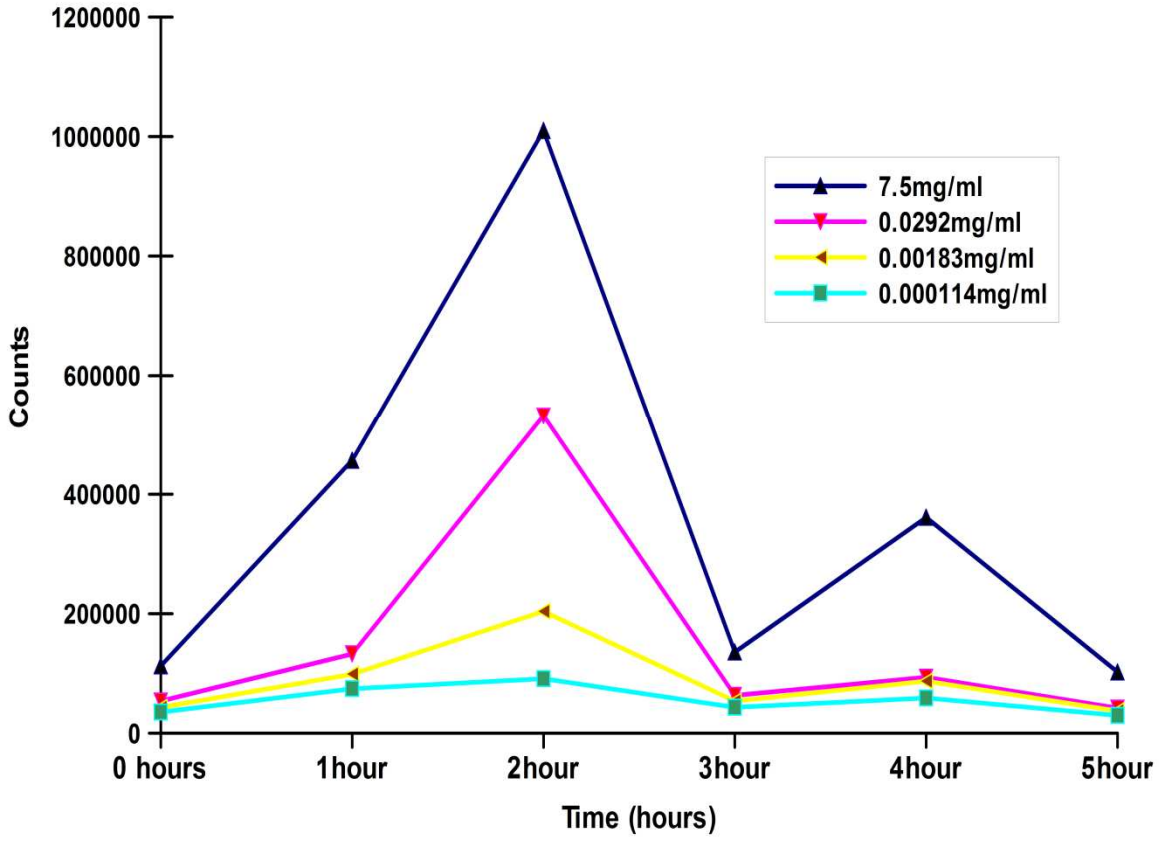

Figure 15 *Bioassay of DPD 2794 with different ciprofloxacin concentrations. Counts are obtained from Chameleon Multilabel plate reader.*

Maximal luminescence range lies in 10^6 for chameleon. This can be seen from figure 15. There is increasing luminescence up to 2nd hour and luminescence starts to decrease up to 3rd hour and its starts to increase and decrease again, this shows the ciprofloxacin's behavior in DPD2794. This two hump phenomenon has to be confirmed with other experiments also. Increase in Luminescence peaked at 2 hours. Since the experiments were dose respondents, the intensities of each sample varied according to the concentrations.

4.2.2 Bioluminescence counting using Wallac Victor^2

Comparison of Photon counting devices with a genotoxic sensor is a new initiative and Victor^2 Wallac is an instrument that is used to compare the behavior of CFX in DPD2794. The Figure 16 below shows the behavior of ciprofloxacin in DPD2794 for a certain period of time.

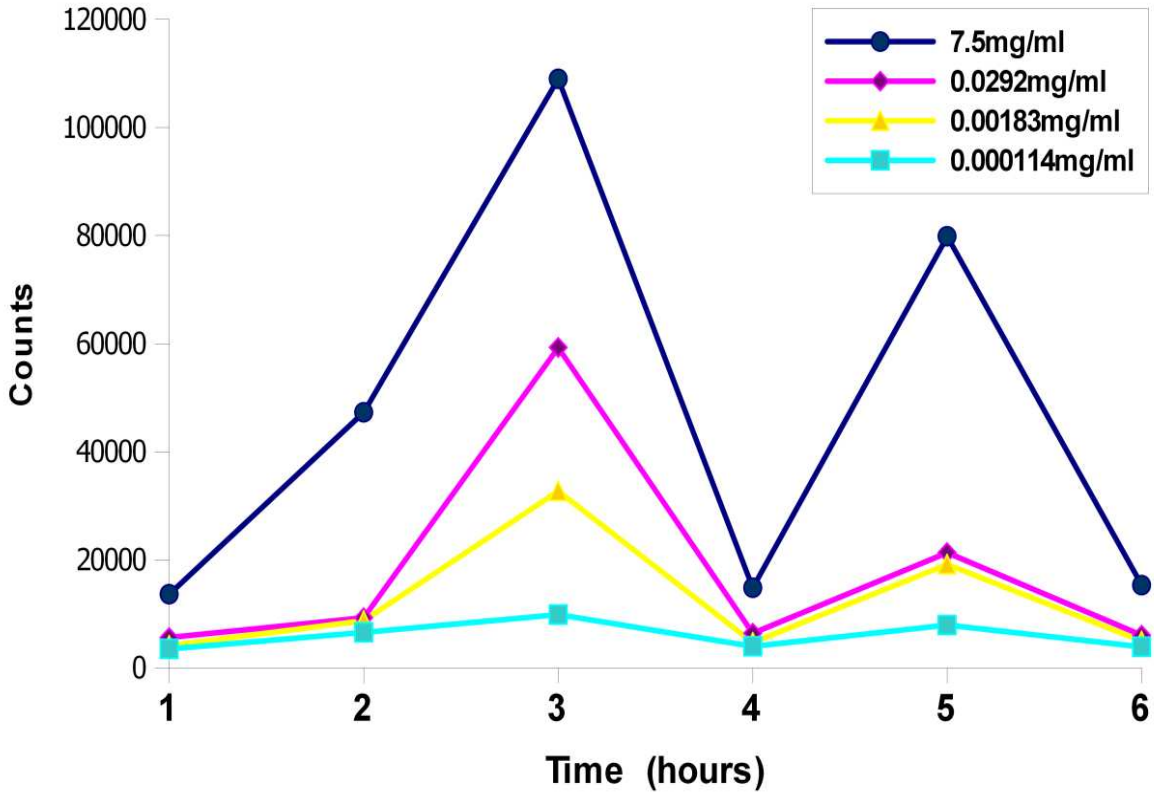

Figure 16 *Bioassay of DPD2794 with different ciprofloxacin concentrations and measurement taken in Wallac Victor^2*

The response to DNA damage sensor was similar (w.r.t. two humps) as mentioned by (Belkin et al., 1997). However, the intensities of Victor^2 Wallac are low in comparison to Chameleon. From figure 13 it can be seen that the intensity peaked at two hours was up to 10^4 counts, whereas the maximum intensity of chameleon at two hours was 10^6 counts. The intensity obtained from a particular machine determines its effectiveness. At four hours the luminescence drops down completely and starts to increase with increase in time. This is called as “lights-off” and “lights-on” mechanism.

4.2.3 Bioluminescent counting using Xenogen Imaging Station

The SOS response to different concentrations of ciprofloxacin in *E. coli* DPD2794 was measured by a bioluminescence imaging system (Xenogen VIVO Vision IVIS Lumina, Caliper Life Science System, USA) using luminescence and image overlay mode with an exposure time of 30 s. and medium binning. Readings were taken with 1 hour intervals for 5 hours. Luminescent zones indicated SOS response. The SOS response was measured as mentioned in (Belkin et al., 1997). To elucidate the differenced produced by differing concentrations, experiments with low to high concentrations (blank, 0.0001, 0.002, 0.03 and 7.5 mg/ml) were compared. As mentioned by Belkin et al., DPD2794 showed strong reaction with differing concentrations.

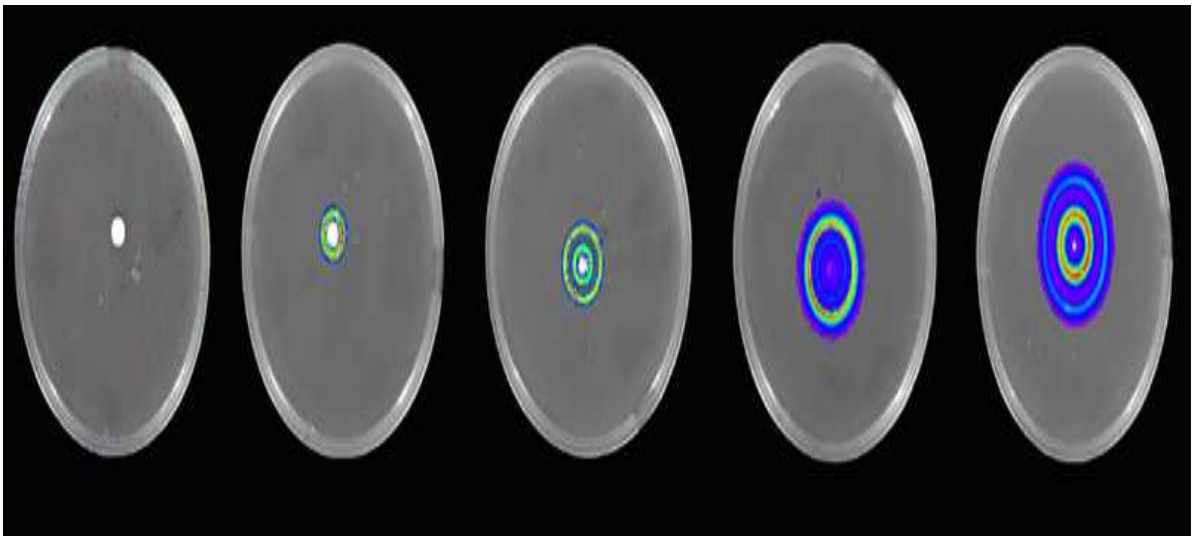

Figure17 *the different concentrations of ciprofloxacin (blank, 0.0001, 0.002, 0.03 and 7.5 mg/ml) showing increased luminescence with higher concentrations of ciprofloxacin. Image taken using IVIS Lumina Xenogen*

Figure 17 shows the luminescent photograph obtained from IVIS Lumina Xenogen. As seen in the picture, luminescence of the sample differs with different concentration. In the picture the concentration are from low to high and the first one is Blank (MQ Water). On the basis of comparison the luminescence emitted from these samples are much lower when compared to the other two luminometer.

Luminometers have photomultiplier tubes, which intensifies luminescence. However, photomultiplier tubes are not present in imaging stations like IVIS Lumina Xenogen (Roda et al., 2009). Nevertheless, Biophotonic imaging station has its own advantage. A comparative micro-titre plate reading was performed as seen in 4.2.1 and 4.2.2, to check the luminescence response with comparison to other photon counters and it was found that luminescence detection range was 10^3 for Xenogen when Chameleon had 10^6 for the same experiment.

In a bio-photonic imaging station the background noise level is very low compared to that of luminometers (Jenkins et al., 2003). This is because of the cooled CCD camera's present inside the black box or in other words Xenogen.

One phenomenon to be noted from the experiments done in other luminometers is the hump phenomenon. Two hump phenomenons were also seen in Xenogen. The figure 18 below shows the clear view of two hump phenomenon, a specific characterization of ciprofloxacin on DPD2794 a DNA damage sensor. For a bioluminescent strain two types of promoters are present, they can be either inducible or constitutive (Lee et al., 2005). This DPD2794 strain has an Inducible promoter.

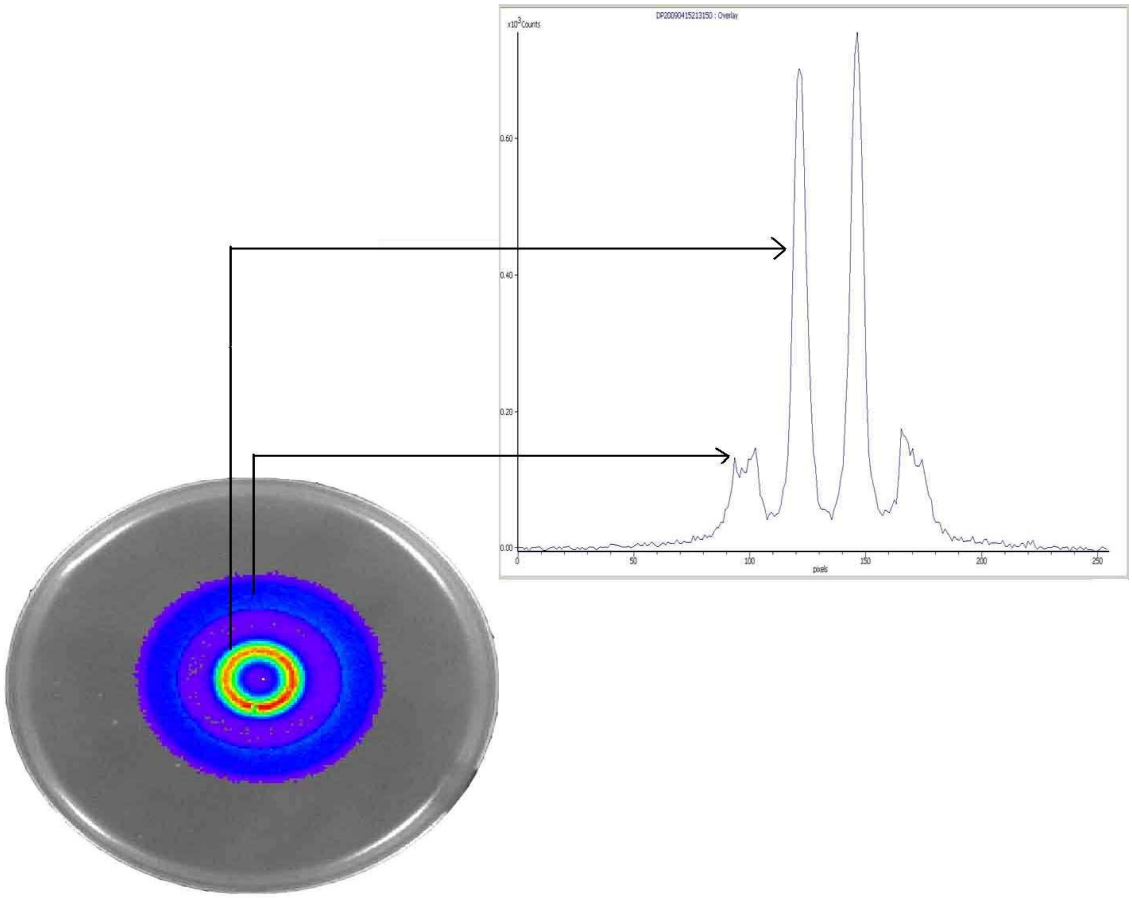

Figure 18 *DPD2794 7.5mg/ml at 5 hours showing the double hump characteristics of ciprofloxacin. A phenomenon that is unique for ciprofloxacin is luminescent photographed in Xenogen*

Strains having inducible promoter show higher level of *lux* genes transcription when the promoter is induced by some specific stimuli as mentioned by (Roda et al., 2009). In this case the specific stimulus is CFX and they induce the promoter to produce luminescence. This specific behavior of ciprofloxacin has to be further studied.

The widths of the circular disc were calculated for 5 hours and the increase in width also showed increase in luminescence. Increase in width was seen for all the concentrations. However, decrease in width could be seen only for certain concentrations. This can be clearly seen below in Figure19.

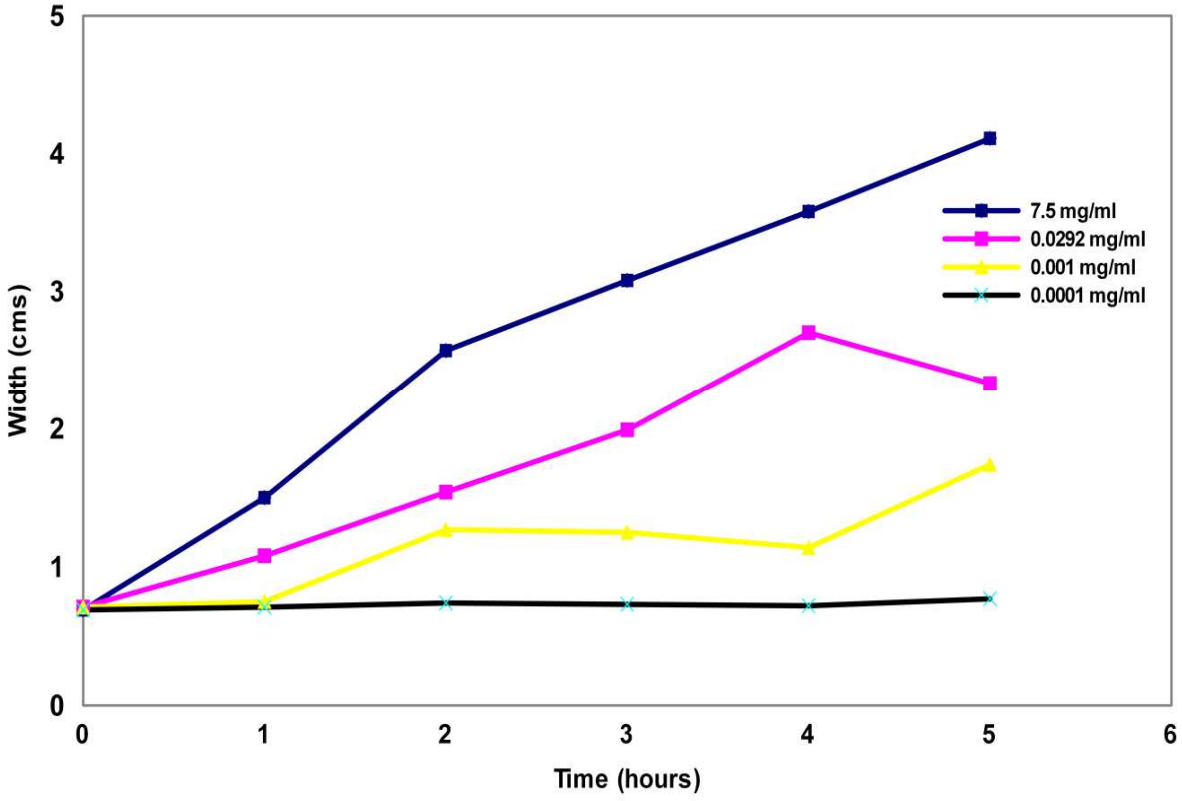

Figure 19 *increase in width of the circle with respect to concentrations of ciprofloxacin*

This could also be because for stronger concentration to revert on an agar plate could take more time than for smaller concentrations. If the experiment was carried out for longer duration then probably reverting could be seen. Nevertheless, any reversion done on an agar plate could not bring a major difference in the width as it is once formed and cannot be completely erased. Whereas, in the case of luminometers, luminescence can fluctuate without any limits, however it depends on the reactive components.

4.3 Direct comparison of three devices using same sensor cells:

Bioassays were aimed at gathering potential light emission from the particular strain responding in a particular pattern for each strain. The luminescence emitted by DPD2794 was considered to be 10fold higher compared to strains of the same category (Belkin et al., 1997). Three Luminescence counting instruments were determined using a microtitre plate. Same amounts of Antibiotic (DPD 2794) and the same *E.Coli* sensor strains were used to directly compare the three luminescence counting instruments. Amounts of bacterial sample and the amount of Antibiotics used were same in all three microtitre plates

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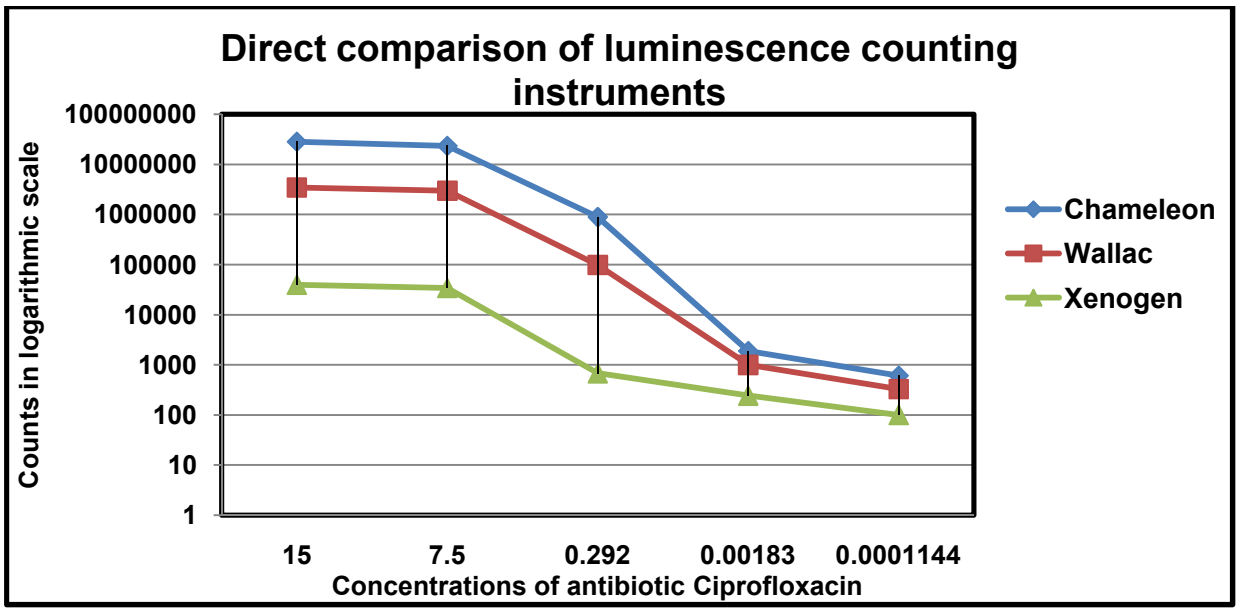

Figure 20 *Direct Comparison of three devices using same sensor cells*

Chameleon Multilabel plate reader, Wallac Victor^2 and IVIS Lumina Xenogen were used simultaneously to determine the effectively of each instruments and also to compare and find out the effective amongst the three Figure 20 show that Chameleon has a higher range luminescence over Wallac Victor^2 and IVIS Lumina Xenogen. The result shows a clear leap of chameleon over Victor^2 wallac and IVIS Lumina Xenogen. Previous comparison of these instruments weren't done together. However, Separate comparisons were done.

The bioluminescence obtained from Chameleon in shown below in figure 21. From this we can incur that the Luminescence Corresponds to the Concentration of the sample used.

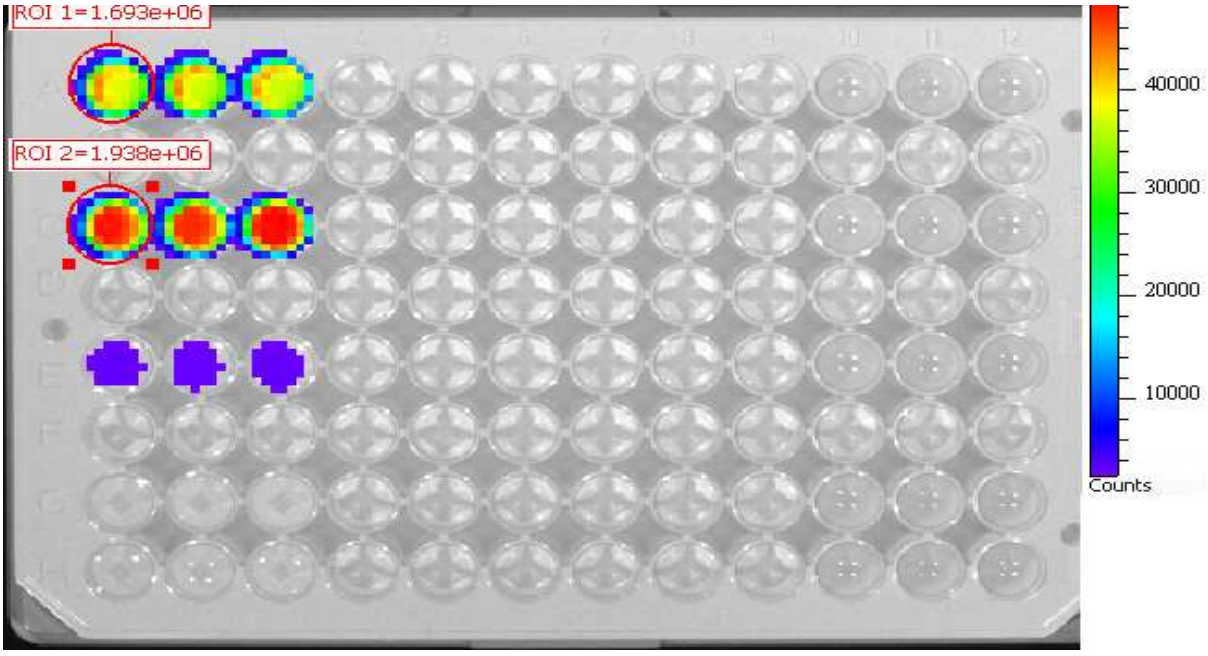

Figure 21 *Showing the luminescence obtained from IVIS Lumina Xenogen*

From Figure 21 it is evident that the luminescent reading obtained from IVIS Lumina Xengoen can be viewed as an Image, this gives Xenogen an edge over Chameleon and Wallac Victor[2]. From all the reading obtained the Background noise were removed by subtracting with the luminescence obtained from the image and interpreted in graph plots.

5 Discussions

5.1 Experimental discussion

All the luminometers used in the comparison study are PC –controlled and allowed the analysis of sample at 37°C.The Performance of three imaging systems was studies for their respective comparison. These comparisons were made as two separate incorporation i.e one with the antibiotics and the other one with arsenic metal sensors. *Escherichia coli* and *Escherichia coli* DPD2794 XL-1(parsRluxCDABE) were used to determine the effectiveness of the luminescence produced from three photon counting devices. The arsenic biosensor strain and whole cell biosensor strain used for luminescence measurements was freeze-dried *Escherichia coli* XL-1 (parsRluxCDABE) and *Escherichia coli* DPD2794. The sensor cells were freeze-dried to diminish batch-to-batch variation and reagent-like usage of the biosensor without tedious culturing of the cells. Suitable stress and temperature conditions were used for the growth of the bacterial strains.The conditions were carefully supervised and maintained at ambient temperature to obtain desired results.

For IVIS agar diffusion assays the arsenic biosensor strain were stored in a 20% glycerol suspension at - 85°C. In the case of Xenogen the temperature inside the Black box is set to be 37°C, so that it equals the incubation temperature of 37°C. Similar adjustments were made with all the incubators and photon readers' in order to obtain same condition I all three photon counting instruments. These experimental similarities help in the direct comparison of all three photon counting devices

5.2 Effectiveness of Arsenate and Arsenite on three luminescence counting devices

Bioluminescence allows for sensitive and rapid measurements of bacterial lux gene expression without the need for incubation on agar plates. Luminescence is expressed as IF (induction factor). The induction factor of Arsenite (4500) being higher than the induction factor of arsenate (4200) at three hours, it is an evidence that arsenite are responding higher compared to arsenate at same concentration levels. Based on the previous studies done by (Hakkila et al., 2002) three hours of readings were done to test the effective luminescence produced in the arsenic biosensor sample. IF from chameleon was taken into note for comparison with other photon counters (Ukonaho et al., 2007).

On comparison with chameleon (luminometer) the sensitivity of Wallac is found to be a lot lesser, which is almost half the times less. The sensitivity of Viictor^2 wallac is a lot lesser than Chameleon. If chameleon could detect luminescence greater than 10^6 times, wallac could detect maximum of 10^5 times for the same sample at same time interval. Although both the luminometers have photomultiplier tubes, the effectiveness of chameleon is profound. Although there are higher applications like flash or glow luminescence, fluorescence, high-sensitivity time resolved fluorescence, flash absorbance and photometry (Mosaddik et al., 2004), the sensitivity is relatively low in comparison to Chameleon.

IVIS Lumina Xenogen has a CCD (Charge coupled Device) Camera, capable of capturing sequences of low-light level images. The camera can be cooled to around -90°C. This is the special advantage of a charge coupled device camera. This helps in the imaging method used for bioluminescence and fluorescence (Jenkins et al., 2003). Fish Samples soaked overnight were examined for duration of 3 hours. From 1.15 hours the luminescent region around the fish started to decrease considerably, which clearly indicated that the luminescent present in the fish is due to the presence of arsenite (III) and arsenate (V). At two hours the luminescence around the *Baltic herring* had decrease very low and the luminescence on the fish kept increasing. Finally at around 3 hours, the luminescence produced by the fishes reached saturation (Xenogen saturation is 60,000 photons), however the region around the fish reduced with time.

Figure 12 shows the periodic increase in luminescence with respect to time for both arsenite and arsenate. From the series of assays performed it is evident that the sensitivity of Xenogen is much lesser compared to that of both the luminometers. However, the visualization of the fish fillet and the pictures are evidence for the change in the luminescence range around the fish fillets. The luminescence change can only be viewed in Biophotonic imaging station like (Xenogen VIVO Vision IVIS Lumina, Caliper Life Science System USA). Selection of a genetic reporter can be difficult due to the increased and wide range of availability of genes. In order to reduce the selection certain methods like luminescence detection can be used (Hakkila et al., 2002) .

5.3 Effectiveness of Ciprofloxacin on *Escherichia coli* as detected by three luminescence counting devices

The recombinant *Escherichia coli* strain DPD2794 containing a *rec*A:*luxCDABE* fusion is used to detect genotoxicity of various chemicals. The same DPD 2794 was used in the experiment to compare the luminescence obtained from three devices. Genotoxic agents were previously categorized into two groups, Direct DNA Damaging (DDD) agents and

Indirect DNA Damaging (IDD) agents.(Min et al., 1999). These strains are also called as genotoxic sensors. They can be used to detect the geno-toxicity of a particular substance

Maximal luminescence range lies in 10^6 for chameleon. This can be seen from figure 15. There is increasing luminescence up to 2^{nd} hour and luminescence starts to decrease up to 3^{rd} hour and its starts to increase and decrease again, this shows the ciprofloxacin's behavior in DPD2794. This two hump phenomenon has to be confirmed with other experiments also. Increase in Luminescence peaked at 2 hours. Since the experiments were dose respondents, the intensities of each sample varied according to the concentrations. Whereas, Victor Wallac produced maximum intensity in the range 0f 10^4, it is comparatively less than that of Chameleon. In the case of infectious disease, the response of the human host and target microbe is activated by the use of Antibiotics and hence the activity of antibiotics on bacteria plays a significant role in the experiment (Yim et al., 2006) done with three specific devices.

The SOS response to different concentrations of ciprofloxacin in *E. coli* DPD2794 was measured by a bioluminescence imaging system (Xenogen VIVO Vision IVIS Lumina, Caliper Life Science System, USA) using luminescence and image overlay mode with an exposure time of 30 s. and medium binning. Readings were taken with 1 hour intervals for 5 hours. Luminescent zones indicated SOS response. The SOS response was measured as mentioned in (Belkin et al., 1997). The detection range for IVIS Lumina Xenogen is Very less compared to that of Chameleon and Victor ^{2} Wallac. However, it gives a clear picture of the antibiotic induced transcription modulation visually. Using antibiotic inducted transcription modulation, luminescence from each plate were converted to the colour scale to indicate the right *lux* expression ((Yim et al., 2006). CFX indicated the presence of bioactiveness.

5.4 Discussion for direct comparison of three devices using same sensor cells

The main objective of this direct comparison of three devices using same sensor cells, from previous studies by Robert Larossa, maintain physiological temperature conditions during measurements is one of the essential conditions for a correct analysis of the activity of cells. These conditions were maintained throughout the experiment.

Bioassays were aimed at gathering potential light emission from the particular strain responding in a particular pattern for each strain. The luminescence emitted by DPD2794 was considered to be 10fold higher compared to strains of the same category (Belkin et al., 1997). DPD2794 a genotoxic sensor which is used to detect DNA damage was used for the direct comparison.

On direct comparison chameleon multilabel plate reader, Victor^2 Wallac from Perkin Elmer life sciences and IVIS lumina Xenogen from caliper life sciences. The maximum range of photon absorbance was seen from chameleon and then Victor^2 Wallac and Xenogen in the Descending order. No previous Direct Comparisons were made with three particular instruments. But from the previous studies ((Hakkila et al., 2002; Min et al., 1999; Tsui et al., 2004; Yim et al., 2006) the data's were proved to be true. The range of Chameleon was higher compared to Victor and Xenogen.

6 Conclusions

This study compares the efficiency, effectiveness and workability of three photon counting devices namely chameleon multilabel plate reader, Victor^2 Wallac a plate reader from Perkin Elmer life sciences and IVIS Lumina Xenogen a biophotonic imaging station from caliper life sciences. This study was successfully studied and their comparisons were done using two bacterial strains *Escherichia coli* DPD2794 and *Escherichia coli* XL-1(parsRluxCDABE). Both the strains employed different promoters in order to employ their scope for detection. The bioluminescence from both the samples was obtained using three different photon counters. Their effectiveness was compared by using genetic engineering technologies and Agar diffusion assay method.

The Obtained bioluminescence was analyzed using a spreadsheet. On a comparative scale, chameleon multilabel plate reader was 10^2times more effective (more luminescence) than Victor^2 Wallac plate reader and 10^3times more effective (more luminescence) than and IVIS Lumina Xenogen. On comparing the efficiency of bioluminescence detected from three different photon counting devices, Chameleon was found to be the highest sensitive and IVIS to be the lowest sensitive. However all instruments had their own advantages and disadvantages.

The role of device background is to obtain the minimum amount of luminescence that is obtained from the imaging system. This background noise has to be finally reduced by calculation to get the appropriate results. However, the background noise differs for Individual instruments. The background noise of Chameleon is 200 photons at initial level, where as it was 40 for Wallac Victor^2. On the other hand the Background for IVIS xenogeny is a lot lesser as it has got CCD camera. CCD camera enables the system to produce lesser background noise.

Working with luminometers required very less technical skill compared to that of Xenogen. Xenogen required processing of the data and calculation of ROI's, width of the sample calculation, brightness contrast adjustments. On the first hand, Couple of things to be noted while working with photon counting instruments. Dilution of samples is a must while working with photon counting devices; however lesser dilution or no dilution is required when working with Biophotonic imaging station IVIS Lumina Xenogen as the agar on the plates makes the total concentration of the toxic and arsenic sample lesser.

Placement of micro titer plates in Chameleon should be carefully handed as the space for placing the micro-titer plate is restricted to little over the size of the micro-titer plates, chances of the sample spilling when the micro-titer plate with sample unloaded from chameleon multilabel plate reader is high.

Working with Xenogen requires a training done by an experienced person and the knowledge about background reading, taken every night is a must as it might determine the automatic background level. An adjustment of the floor of the Xenogen, depending upon the type of plates used is a must.

Luminometers are easy to use and handle. Multiple concentrations can be run at a time and it is quicker. Photo multiplier tubes present in them intensifies the signal obtained from the sample. On the other hand Biophotonic imaging station is a novel approach with CCD detection technology that enables the study of assays, liquid cultures, solid samples and live animals. Finally from the experiments carried on I can conclude that the luminescent detection efficiency of chameleon multilabel plate reader is the highest, followed by Victor^2 Wallac and Xenogen with the least efficiency of the three.

From 4.3 it can be directly inferred that the photons absorbed by Chameleon is more compared to that of Victor^2 wallac and IVIS Lumina Xenogen. On a comparative scale, chameleon multilabel plate reader was 10^2times more effective (more luminescence) than Victor^2 Wallac plate reader and 10^3times more effective (more luminescence) than and IVIS Lumina Xenogen.

When the Exposure time was increased, there seemed to be more luminescence in the plate and more background noise. This is because more time was allowed for the photon readers to take the luminescence from the plate. Henceforth from the previous knowledge, standards and optimum exposure times were set for all three instruments. Imaging of whole microtitre plate at once gives us the idea that there is luminescent reaction taking place and also the range of luminescence present in the plate. Based on the Luminescent image from imaging station, the addition mixtures like antibiotics are biosensors can be added in adequate amount for desired results.

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